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LIPOID PHOSPHORUS OF WHEAT AND ITS DISTRIBUTION

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In previous communications from this laboratory, it has been shown that the lipoid content of glutens from different grades of flour milled from the same wheat is highest in the gluten of lowest quality (Sullivan and Near, 1927a, 1927c).

Also, it has been found that the lipoid content of wheats of widely different variety and quality varies within relatively narrow limits (2.53–3.84% in twenty wheats examined) and that the lipoid-protein ratio gives a valuable indication as to the quality of wheat, the softer, weaker grain showing the higher ratios.

It was thought that, as an extension of these studies, it might be of interest to measure the percentage of phosphorus found in the lipoids extracted from different parts of the wheat kernel. The phosphorus percentage can then be taken as an indication of the amount of phosphatides, such as lecithin, present in the various grades of flour, as the other substances extracted by alcohol and ether do not contain phosphorus.

Historical

Töpler (1861) estimated the amount of lecithin present in various seeds and other parts of plants. He extracted the tissue with ether, estimated the phosphorus in the extract, and calculated the percentage of lecithin from the amount of phosphorus found. Beyer (1871), and later Jacobson (1899), showed that ether extraction alone was unsuitable for quantitative measurements of this nature. Jacobson extracted seeds with alcohol, estimated the phosporus in the ether-soluble part of the alcohol extract, and obtained much higher results than previous investigators. It has since been proved that part of the phosphatides in both plant and animal organs exists in a combination with other substances, probably proteins. This combina-

tion is insoluble in ether and must first be dissociated by hot alcohol before the extraction is complete.

Experimental

A hard spring wheat of the Marquis variety ,grown near Valley City, North Dakota, and its milled products were taken for analysis. Previous to milling in a 1200-barrel mill, the wheat was thoroly washed. The percentage yield of the flours and feeds is given in Table I. Phosphorus was determined volumetrically according to the official method of the A. O. A. C.

TABLE I

ANALYSES OF A MARQUIS WHEAT AND ITS MILLED PRODUCTS
(Calculated to dry basis)

	Yield	Ash	Nitrogen	Lipoid	Ether extract	Lipoid-ether extract x 100 / Lipoid
	%	%	%	%	%	
Patent	58.0	0.464	2.49	1.79	1.15	35.75
Clear	12.0	0.778	3.16	2.44	1.88	22.95
Wheat	....	1.855	2.90	3.02	2.30	23.90
Low grade	2.6	1.647	3.41	4.39	3.51	20.01
Total mill run middlings	14.4	4.637	3.43	7.29	6.25	14.26
Bran	13.0	6.497	3.25	5.74	4.76	17.07
Germ	*	4.628	5.13	12.04	10.81	10.21

*The germ, which would have a percentage yield of less than 1 per cent, was diverted to the middlings and bran and is included there. The sample of germ, analysis of which is given, was taken from the spouts before it reached the middlings and bran.

In all cases the sample (0.5–2 gm. depending on the amount of phosphorus) was digested by heating for a few hours in a Kjeldahl pyrex flask with 25–50 cc. H_2SO_4 and 0.5 gm. mercury until the solution was colorless and the acid had evaporated to a very small volume. The sample was boiled with distilled water and the solution neutralized with ammonia; 5–10 cc. conc. HNO_3 and 15 gm. NH_4NO_3 were then added and the solution was heated to 50°C. and the phosphorus precipitated as ammonium phosphomolybdate. The yellow precipitate was dried at 110°C. for 2 hours, weighed, and the result checked with that obtained by dissolving the ammonium phosphomolybdate in standard NaOH and titrating with standard acid. In some instances these two results were also checked on the ash of the flours and feeds by the standard gravimetric procedure weighing the phosphorus of the ash as $Mg_2P_2O_7$. The phosphorus in the lipoid material was determined as described above.

After drying in vacuo, 0.6–0.8 gm. of lipoid was taken up with ether, the ether evaporated over a water bath, and the organic

material destroyed by heating with sulfuric acid, 0.5 gm. mercury and 2 gm. KNO_3. When H_2SO_4 is used for the destruction of organic matter in a phosphorus determination, it is important that as little as possible be left at the end of the digestion, and that sufficient HNO_3 and NH_4NO_3 be added. Without these conditions, high results are often obtained.

The direct extraction method employed to attain the lipoids of the products was essentially the same as the procedure recently outlined by the authors (1927a). It has been found, however, that the same results are obtained if the time of the ether extraction is shortened, as practically the entire amount of lipoids is removed in the first extraction with hot alcohol. The alcohol extraction was therefore extended from one-half hour to 1 hour and the ether refluxed for one-half hour instead of 2 hours as formerly recommended. The ether is of value in cleaning out some of the material left in the cotton and the extraction cylinder. A 6–8 gm. sample was mixed with 5 gm. of pumice stone and extracted in an alundum cylinder of porosity R A 98. For the first extraction, 100 cc. of 95% alcohol was used, followed by 100 cc. of ethyl ether. No ammonia was used with the alcohol. Identical results, however, are obtained by the use of the alcohol and ammonia mixture and the alcohol alone.

Discussion

The term lipoids is used, as in previous articles by the authors, to designate the alcohol–ether extract of wheat and its milled products. This extract includes not only phosphatides but also neutral fats, small amounts of fatty acids, chlorophyll, and sterols.

Anderson and Nabenhauer (1924) have shown that wheat endosperm contains at least two different sterols, an unsaturated sterol, sitosterol, $C_{27}H_{45}OH$, and a saturated compound dihydrosterol, $C_{27}H_{47}OH$. Bran was found to be particularly rich in the saturated sterol.

The total phosphorus in all the products is given in Table II and also the phosphorus contained in the lipoid material and ether extract. Altho the highest grade of flour, the patent, has the least total phosphorus of all the products, it contains more of this element in its lipoid than do any of the lower grades. The percentage of phosphorus contained in the lipoids decreases with decreasing refinement of flours from which they were extracted, while the per-

centage of lipoids and total phosphorus increases. For additional information, the phosphorus of the ether extracts of all samples was also determined. A much lower percentage of phosphorus is obtained from ether than from alcohol extracts. There is a very small range, 0.0822%, of the phosphorus percentage in the ether extracts of the various products, altho the percentage of phosphorus was also highest in the patent and clear and lowest in the bran and germ. In the lipoids, the maximum variation is 0.3486%. For the quantitative estimation of phosphatides such as lecithin, in wheat or its products, it is evident that extraction with ether is unsuitable—in some cases the amount of phosphorus in the ether extract being only half that found in the alcohol extract. This is true of most plant and animal organs.

TABLE II

PHOSPHORUS CONTENT OF PRODUCTS, LIPOIDS, AND ETHER EXTRACTS
(Calculated to dry basis)

	Total phosphorus of product	Phosphorus in lipoid	Phosphorus in ether extract	Lipoid phosphorus	Ether phosphorus
	%	%	%	%	%
Patent	0.0988	0.7079	0.3356	0.0127	0.0038
Clear	0.1713	0.5550	0.3038	0.0136	0.0057
Wheat	0.4182	0.5666	0.2578	0.0171	0.0059
Low grade	0.3479	0.4444	0.2686	0.0195	0.0094
Total mill run middlings	0.8954	0.4331	0.2937	0.0316	0.0184
Bran	1.393	0.4346	0.2534	0.0249	0.0121
Germ	1.083	0.3593	0.2839	0.0433	0.0307

In Table II is also given what is termed the lipoid phosphorus. This result was calculated by multiplying the percentage of total lipoid in the product by the percentage of phosphorus contained in the lipoid and consequently is the amount of total phosphorus in the product which is a constituent of the lipoid. The ether phosphorus was determined in the same way. The lipoid phosphorus is naturally lowest in patent flour, increasing with decreasing refinement following the order of their lipoid contents. The percentage of nitrogen in the lipoids extracted from the patent flour and also from the embryo of the wheat was measured. In the patent lipoid it was 1.008% and in the germ, 0.39%. This indicates that the nitrogen percentage of the lipoids is in the same order as the phosphorus content, that is, that patent flour, altho having the least total nitrogen, contains more of this element in its lipoid than do the lower grades.

In Table I are given the analyses of the different separations of wheat as practiced in commercial milling.

As shown in a previous article (1927a), lipoid content increases with decreasing refinement. Table I shows the increase in percentage found in the lipoid extraction over ordinary fat extraction. In these particular samples the alcohol, even in the case of the feeds, extracted more than anhydrous ether when used alone, whereas it was previously found that the action of ether alone on the feeds gave results which were as high as the lipoid percentage. The lipoid percentages in the flours were found to be higher than the corresponding ether extracts, in this case 25–55%. In the samples listed in Table I, the ether dissolves more in the feeds in relation to the total amount of lipoids present than in the flour. The percentages can be seen in Column 6, Table I. This ratio is 35.75% in the patent flour, decreasing progressively with the lower grades to 10.21% in the germ. This signifies that the lipoid material in the feeds contains, in proportion, more neutral fats and other ether-soluble material than the flours. The phosphorus in the lipoid decreases in exactly the same order as the ratio

$$\frac{\text{Lipoid—ether extract}}{\text{Lipoid}}$$

Some interesting ratios calculated from the data in Table II are given in Table III. The ratio $\frac{\text{Lipoid phosphorus}}{\text{Total phosphorus}}$ is highest in the flour of the highest quality and decreases toward lower grades. The ratio $\frac{\text{Phosphorus in lipoid}}{\text{Total phosphorus}}$ goes in the same direction. The ratio $\frac{\text{Lipoid phosphorus}}{\text{Total nitrogen}}$ is lower in the flours than in the total mill-run middlings, bran, and germ.

TABLE III

RATIOS CALCULATED FROM TABLES I AND II

	$\frac{\text{Lipoid phosphorus}}{\text{Total phosphorus}}$	$\frac{\text{Lipoid phosphorus}}{\text{Total nitrogen}}$	$\frac{\text{Phosphorus in lipoid}}{\text{Total phosphorus}}$	$\frac{\text{Total nitrogen}}{\text{Total phosphorus}}$
	%	%	%	%
Patent	0.1280	0.0051	7.16	196.0
Clear	0.0794	0.0043	3.24	232.3
Wheat	0.0409	0.0059	1.35	170.0
Low grade	0.0560	0.0057	1.28	174.9
Total mill run middlings	0.0353	0.0095	0.48	108.5
Bran	0.0179	0.0077	0.31	130.5
Germ	0.0400	0.0084	0.33	118.4

The percentage of lecithin in the wheat products, as calculated from the percentage phosphorus found in their lipoids, is higher than that reported by other investigators.

Summary

In an analysis of the lipoids extracted from various parts of the wheat kernel, it has been found that the highest percentage of phosphorus is present in the lipoid from the highest grade of flour (the patent) which contains the least total phosphorus. The phosphorus percentage of the lipoids decreases with decreasing refinement and increasing total phosphorus of the products from which they are extracted. The nitrogen content of the lipoid extracted from the patent was higher than of that extracted from the germ. Anhydrous ether extracts a smaller amount of the total lipoids in the separations from the endosperm than from the embryo and pericarp of the grain.

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STUDIES OF THE EFFECT ON THEIR BREAD-MAKING PROPERTIES OF EXTRACTING FLOURS WITH ETHER[1]

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Working (1924) has shown that the addition of certain lipoids to flours injures their bread-making properties, and that wheat phosphatide and egg lecithin have a particularly harmful effect on the quality of the gluten as well as on the baking properties of the flour. When 3% of wheat phosphatide was added to the flour, the crude gluten could not be recovered because of its lack of tenacity. Further evidence of the harmful effect of the phosphatides on the flour proteins was shown by the fact that the viscosities of the flour-water suspensions containing added phosphatide were always lower than those of similar suspensions containing no added phosphatide.

In their investigations on the chemical constituents of gluten which influence its quality, Sullivan and Near (1927a) found that glutens washed from a whole wheat and a clear flour were of higher lipoid content than those from patent flour. As the gluten from the patent was of better quality than that from the lower grade flours, they ascribed the poorer quality of the latter to the higher lipoid content. In a later paper, Sullivan and Near (1927b) stated that while there was little variation in the lipoid content of the wheats which they investigated, there was a considerable and significant difference in the ratio of lipoid to protein. They therefore concluded that this ratio gave valuable information as to the character of the gluten and the quality of the wheat. The higher ratios of lipoid to protein were encountered in the softer wheats.

As flours of high lipoid content were found to be of relatively poorer quality for bread-making purposes, and as the addition of such lipoids as wheat phosphatide or lecithin resulted in further impairing the quality of flour, it appeared possible that flour might be improved by extracting it with ether. The major part

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of the material extracted from wheat with ether is true fat, i.e., glycerides of the fatty acids. There are also extracted, however, smaller amounts of substances soluble in fat or in ether, such as phosphatides, sterols, pigments, glycolypins, sulpholypins, and other less familiar substances. It is recognized that simple extraction with ether will not completely remove all the substances in flour which are usually classified as lipoid. Rask and Phelps (1925a, 1925b) have shown that ether alone did not completely extract all the lipoids from cereal products. In order to determine the total quantity of lipoids, these workers found it necessary to subject the flour to several extractions with hot ammoniacal alcohol followed by extractions with ether. Such treatment obviously would ruin the flour for bread-making purposes, and as it was desired to work with the flour after the extraction of the lipoids, such a procedure was not possible. The data of Rask and Phelps show that ether removed about 80% as much fatty material as hot ammoniacal alcohol followed by ether. Hence, even tho extraction with ether may not have completely removed the lipoids, if the lipoids did exert deleterious effects on the bread-making quality of the flour, enough were removed that the extracted flour should show improvement in baking quality, assuming that the ether itself had no harmful effect.

As early as 1908, Salamon reported experiments which proved that the fatty matter of the flour exercised an enormous influence upon the size of the loaf. He treated large samples of flour with ether in order to extract the fatty matter. If a loaf were made from the flour free from fatty matter in the same way as a similar loaf from flour containing the fatty matter, the former was much larger and of more desirable texture than the latter. Winton (1911) found that ether-extracted flour could be baked into bread of as good volume and texture as unextracted flour, but that the bread from the extracted flour lacked the rich "nutty" flavor which characterized that from the natural flour, altho the latter was much whiter than the former.

Stockham (1920) stated that extraction of flour with ether impaired the baking quality, a smaller loaf volume resulting than with the original untreated flour. He also found baking strength improved by the addition of a small quantity of wheat fat to the original flour. The addition of certain other fats appeared to impair the bread-making properties. Saunders, Nichols, and Cowan (1921) found, on the other hand, that the addition of such

fats as lard, butter, and vegetable oils improved the bread. Herman and Hart (1927) found that crumb color, grain texture, and crust color of bread were improved by the addition of increasing quantities of fat represented as "hydrostearoleine." Loaf volume was not materially affected by the addition of 1 and 2 per cent of the fat, but was decidedly decreased by the addition of 3 per cent. It is not the purpose of this work to investigate shortening agents, however, but rather to study further the effects on bread-making properties of the lipoid substances occurring naturally in the flour.

Experimental

In connection with studies on wheat oil reported by Ball (1926), approximately 25-pound portions of flours from Canada, Montana, Minnesota, and Nebraska were extracted for 72 hours in a Lloyd extractor. The ether remaining in the flour after extraction was vaporized at room temperature and the ether-extracted flours listed in Table I were thus obtained. Equal quantities of the natural flours were saved for comparison with those extracted with ether. The appearance of the ether-extracted flours was quite characteristic. As noted by Winton (1911), such flour resembled powdered starch, not only in color but in physical condition. Altho its moisture content was as high as that of natural flours, it was loose, lacked coherence, and could not be slicked for pekarizing.

TABLE I

BAKING AND ANALYTICAL DATA ON NATURAL AND ETHER-EXTRACTED FLOURS MILLED FROM WHEATS GROWN IN DIFFERENT PARTS OF THE UNITED STATES AND IN CANADA*

No.	Source of flour	Treatment	Ash	Protein, dry basis	Loaf vol.	Color score	Texture	Absorption	Gluten		Chopin extensibility value	Viscosity 21 gm. flour-water conc.
									dry	wet		
			%	%	cc.			%	%	%		°MacM.
1	Montana	Natural	0.42	13.87	1680	96	95	58.8	42.1	13.0	25.4	215
2	Montana	Ether-extracted		13.82	1660	99	99	58.8	41.2	12.8	20.2	220
3	Canada	Natural	0.41	13.78	1700	95	94	63.3	41.9	11.6	23.6	216
4	Canada	Ether-extracted		13.55	1820	99	97	64.1	42.0	11.3	19.4	225
5	Minnesota	Natural	0.46	11.74	1680	95	93	57.7	34.2	11.6	22.0	140
6	Minnesota	Ether-extracted		11.44	1780	99	97	58.8	34.1	11.8	20.1	140
7	Nebraska	Natural	0.43	12.75	1500	95	94	61.1	36.0	11.2	21.0	121
8	Nebraska	Ether-extracted		12.16	1520	99	98	61.1	34.2	10.6	19.0	126

*The bread shown in Figure 1 was baked from these flours.

The baking data and other data obtained from a study of these flours are given in Table I. Ash determinations were not made on the ether-extracted flours, as there appeared to be no reason for these flours to be significantly different in ash content from the corresponding natural flours. The protein content (i.e., nitrogen x 5.7) of the extracted flours was found in every case to be slightly lower than that of the natural flours. This is no doubt due to the extraction of certain nitrogen-containing lipoids. Greaves (1911) also noted that the extraction of flours with ether resulted in slightly lowering their nitrogen content.

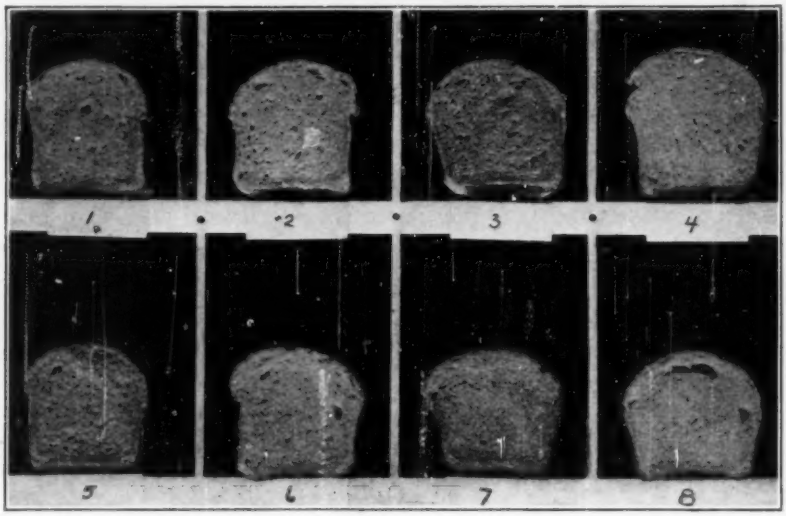

Fig. 1. Bread Baked from Natural and Ether-extracted Flours Described in Table I

1. Montana natural flour. 2. Montana ether-extracted flour. 3. Canadian natural flour. 4. Canadian ether-extracted flour. 5. Minnesota natural flour. 6. Minnesota ether-extracted flour. 7. Nebraska natural flour. 8. Nebraska ether-extracted flour.

In every case the bread produced from the ether-extracted flour was superior to that produced from the corresponding natural flour. The loaf volume of bread produced from natural and ether-extracted Montana and Nebraska wheat flours was within experimental error of being the same, while the volume of the bread produced from the extracted Canadian and Minnesota flours was significantly greater than that from the natural flours. Thus the loaf volume of the bread produced from the natural Canadian flour was 1700 cc. while that from the same flour extracted with the ether was 1820 cc. The improvement in color and texture of the bread due to extraction with ether was much greater than the improvement in volume. In all cases the color and texture of

the bread produced from ether-extracted flours were three or four points better than of the breads from the corresponding natural flour. The bread produced from the natural Montana flour had a color score of 96, while that produced from the same flour extracted with ether had a color score of 99. In the same way the texture scores of bread produced from the natural and the extracted flours are 95 and 99 respectively. The improvement in volume, color, and texture as a result of ether extraction is well shown in Figure 1. The odd-numbered loaves were baked from natural flour, while the even-numbered loaves were from the same flour after extraction with ether.

In absorption and in the wet and dry gluten percentages, as given in Table I, no consistent differences appear between the natural and the ether-extracted flours. It was thought that if the extraction with ether had impaired or improved the gluten it would appear in the quality or the quantity of gluten. No differences were noted in the quality of the gluten, and, as already stated, the values in Table I show no significant differences in quantity.

The flours were further studied by subjecting them to tests with the Chopin extensimeter. Table I shows that in every case the extensibility of the natural flour was greater than that of the flour extracted with ether. Extensibility determinations were conducted as were those of Bailey and Le Vesconte (1924). The dough used for measuring extensibility is allowed to stand for 30 minutes before making the determination. Considerable time was required for the ether-extracted flour to become hydrated. It is possible that if more time had been allowed for the flour particles to hydrate, a smoother dough would have been formed and the extensibility would be more closely correlated with the better bread produced from the ether-extracted flour.

The viscosity of extracted acidulated suspensions of these flours was also determined, Gortner's method (1924) being used. The data in Table I show that there was no significant difference between the viscosity of natural flour suspensions and that of ether-extracted flour suspensions. Working (1924) noted that when flour suspensions were prepared for the viscosity determination according to Gortner's method, one of the reasons for the increase in viscosity on extraction was that water removed not only electrolytes which depressed hydration when the suspension was acidulated, but also phosphatides which decreased the viscosity by the introduction of surface films. It is interesting to note that

the viscosity was approximately the same whether the phosphatides were extracted from the flour first with ether and then with water, as for the ether-extracted flours, or whether the natural flours were extracted with water in the usual way. It appears, therefore, that so far as affecting the viscosity of extracted acidulated flour-water suspensions is concerned, the phosphatides of natural flours are just as completely removed by extraction with water alone as by extraction with ether followed by extraction with water. That water alone should remove the phosphatides from flour as completely as ether, is hardly to be expected. This will be investigated further.

TABLE II

BAKING AND ANALYTICAL DATA ON A NATURAL FLOUR* AND ON THE SAME FLOUR TREATED IN DIFFERENT WAYS

Flour No.	Treatment	Loaf vol.	Color score	Texture	Absorption	Gluten		Chopin extensibility value	Viscosity 12 gm. flour
						wet	dry		
		cc.			%	%	%		°MacM.
1	Natural	1840	97	96	62.2	40.8	13.4	24.0	46
2	Natural + 5% lard	1800	97	96					
3	No. 1 ether-extracted	1820	99	99	62.2	41.0	14.0	22.0	46
4	No. 3 + 5% lard	1800	99	99					
5	No. 3 + 10% lard	1740	99	99					
6	No. 1 treated with 96% alcohol	1660	96	93				13.0	20
7	No. 1 treated with 70% alcohol	1200	95	92				10.0	8
8	No. 1 treated with water	1860	97	97				17.2	30

*The ash and protein percentages of this flour were 0.53 and 13.96 respectively.

In order to learn something concerning the mechanism by which the extraction with ether improved the flour for bread making, another flour was ether-extracted and lard added to both the natural flour and the ether-extracted flour during the bread-making process.[2] The results are given in Table II and the bread is shown in Figure 2. Again the bread from the ether-extracted flour was decidedly superior to that from the natural flour in both color and texture. There was no significant difference in volume. The use of lard, even in the relatively large quantities used in this work, did not lower the texture of the bread from the ether-extracted flour to that of the bread from the natural flour. In accordance with the results of Herman and Hart (1927), the lard tended to reduce the loaf volume. The facts that the addition of

[2] The baking test described by Bailey (1916) was used. Unless otherwise specified, no lard or shortening was used in the formula.

lard did not reduce the color and texture score of the bread baked from either natural or ether-extracted flour and that ether extraction of the flour notably improved the bread, indicate that some ether-soluble material other than true fat is responsible for variations in flour strength. The research of Working (1924), in which phosphatides were shown to influence the quality of bread, is the only work known in which an effort was made by experiments to associate the bread-making properties of flour with a single type of the substances in the ether extract or in the lipoids (defined according to Rask and Phelps, 1925a).

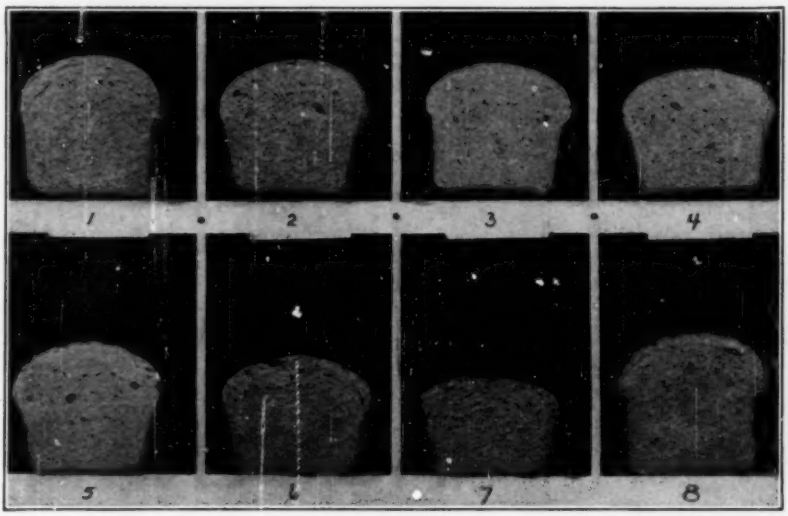

Fig. 2. Bread Baked from Natural and Ether-extracted Flours Described in Table II

1. Natural flour. 2. Natural flour +5 per cent lard. 3. Flour 1 extracted with ether. 4. Flour 3+5 per cent lard. 5. Flour 3+10 per cent lard. 6. Flour 1 treated with 96 per cent alcohol. 7. Flour 1 treated with 70 per cent alcohol. 8. Flour 1 treated with water.

A portion of the same flour used in the last work was doughed with 96% alcohol, another portion with 70% alcohol, and a third portion with tap water. The doughs were pulled into small bits and dried at room temperature with an electric fan. The dried dough was then ground on the smooth rolls of an experimental flour mill in order to get rid of all lumps and to obtain flours of uniform fineness. The flours thus obtained were baked into the bread represented as 6, 7, 8, in Figure 2. The data are given in Table II and show that the treatment with 70% alcohol was most injurious to the bread-making properties of the flour, treatment with 96% alcohol was less injurious, while treatment with water was least injurious. Sharp, Gortner, and Johnson (1923) found

little difference in the effect of treatment with 70% and 96% alcohol on the bread-making properties of the flour with which they worked. It is possible that unknown differences in the treatment of the flours may account for the differences in baking results.

Viscosity and extensibility determinations were also made on flours treated with alcohol and with water. By every test the treatment with 70% alcohol produced the more deleterious results. Loaf volume decreased from 1840 cc. for natural flour to 1300 cc. for that treated with 70% alcohol; the viscosity decreased from 46 to 8 degrees MacMichael; and the extensibility decreased from 24 to 10. Treatment with 96% alcohol lowered the extensibility to 13, and treatment with water lowered it to 17.2. The viscosity of the flour treated with water was reduced from 46 to 30 degrees MacMichael, while that of the flour treated with 96% alcohol was decreased to 20 degrees MacMichael. From these data it appears that treatment with 70% alcohol was the most injurious, treatment with 96% was less injurious, and treatment with water was least injurious.

As Working (1924), and Sullivan and Near (1927) suggested that a relationship exists between the lipoid content of flours and flour strength; and as low-grade flours are known to be high in lipoid content, a patent, a first clear, a second clear (all milled from the same wheat), and a durum flour were ether-extracted with the idea in mind that if the lipoids played an important rôle in flour strength, the improvement due to extraction with ether would be greatest for the low-grade flours, from which the largest quantities of lipoids would be extracted. The flours were extracted for 72 hours in a Lloyd extractor and treated as already described for the flours listed in Table I. Results are given in Table III. The baking data indicate again that extraction with ether markedly improved the color, texture, and volume of the bread. Also that the improvement, especially in loaf volume, appears to be most marked for the low-grade flours. Bread baked from the ether-extracted patent flour was 40 cc. larger than the natural patent flour bread; the ether-extracted first clear, 80 cc. larger than the natural first clear; the ether-extracted second clear, 120 cc. larger than the natural second clear; and the extracted durum flour bread, 110 cc. larger than the natural durum flour bread. These represent increases in volume due to ether extraction of 2.4, 5.1, 10.7, and 7.0% for the respective breads. It is evident that the greatest

improvement occurred in the second clear flour. Ether extraction of low-grade flours, however, did not increase the loaf volume of the breads produced from them to that of the patent flour milled from the same wheat.

TABLE III

BAKING AND ANALYTICAL DATA ON NATURAL AND ETHER-EXTRACTED FLOURS OF DIFFERENT GRADES

Except for the durum flour, the flours were obtained from the same wheat.

Flour grade	Treatment	Ash	Protein dry basis	Loaf vol.	Color score	Texture	Absorption	H-ion conc. of flour	Reducing sugar per 10 gm. of flour	Diastatic activity, Rumsey units
		%	%	cc.			%	as pH	mgm.	
Patent	Natural	0.43	11.92	1680	98	100	59.7	5.92	38	269
Patent	Ether-extracted	0.44	11.95	1720	100	104	60.2	5.97	48	304
First clear	Natural	1.13	14.56	1560	93	95	60.6	6.34	40	223
First clear	Ether-extracted	1.16	14.70	1640	95	100	60.0	6.17	60	284
Second clear	Natural	1.67	14.27	1120	..	...	62.2	6.46	60	290
Second clear	Ether-extracted	1.76	14.54	1240	..	...	63.3	6.23	108	401
Durum	Natural	0.76	13.21	1500	96	94	62.6	6.34	60	246
Durum	Ether-extracted	0.80	13.42	1610	99	96	62.6	6.40	92	270

The reducing sugar content and the diastatic activities of the natural and ether-extracted flours were also determined. The data in Table III show that ether-extracted flours were always higher in reducing sugars and in diastatic activities than the corresponding natural flours, the second clear flour showing the largest increase. Thus the reducing sugar content of the natural flour was equivalent to 60 mgm. of maltose per 10 gm. of flour, while after extracting with ether it was 108 mgm. Similarly, the diastatic activity (in Rumsey units) of the natural flour was 290, while after extraction with ether it increased to 401.

In an effort to explain the reason for the changes in diastatic activity, the H-ion concentration of the suspensions of the natural and ether-extracted flours was determined. The first and second clear ether-extracted flours yielded suspensions which were more acid than those of the natural flours, but the opposite was the case for the patent and the durum flours. Hence, while changes in H-ion concentration due to ether extraction may have some influence in changing the diastatic activity of the flours studied, it can not have been the only factor. As the flour particles were much finer after extraction, it is suggested that the starch in the natural flour may exist in relatively large clumps of granules held together by fatty material. When this fatty material is removed the clumps fall apart, thereby forming a more suitable substrate

for the diastase. The author, in collaboration with R. C. Sherwood, observed (unpublished data) that modifying the size of flour particles may result in a substantial change in diastatic activity. The suggestion that the removal of fatty material from flour allows the clumps of starch to fall apart also explains the higher reducing-sugar content of the extracted flour as compared with the natural flour. The clumps of starch may occlude reducing sugars which are extracted for determination only when the occluding material falls apart.

Discussion

In order to determine the effect on bread-making properties of the ether-soluble substances, nine flours were extracted with ether, and bread was prepared from both the extracted and the natural flours. Flours milled from wheats grown in Montana, Canada, Minnesota, and Nebraska were used, as well as a patent, a first clear, and a second clear, all milled from the same wheat. A durum flour was also extracted. In all cases the bread produced from the extracted flours was superior in color and texture to that produced from the natural flour, and in most cases the loaf volume was greater. Even after flours were stored for two years, the ether-extracted flours still produced better bread than the natural flours.

When quality and quantity of gluten, absorption, and viscosity of extracted and of natural flours were compared, no significant differences were found. The extensibility of the extracted flours, as measured by the Chopin apparatus, was smaller in every case for the extracted flour than for the corresponding natural flour. This is not in accordance with what might be expected, since, as pointed out by Working (1924), the presence of the phosphatide should reduce the tenacity of a dough, hence its removal should increase the tenacity. It took considerable time for the flour particles in the dough prepared from ether-extracted flour to become completely hydrated, and it is thought that by taking this into account and modifying the procedure for measuring the extensibility accordingly, results more in line with the baking results might be obtained.

The diastatic activity and the original reducing sugar content were greater in every case for the ether-extracted flours than for the natural flours. It is interesting to note that the reducing sugar content and diastatic activity resulting from extraction with

ether both increase as the grade of flour decreases, the former being 10 mgm. per 10 gm. of flour for the patent flour; 20 mgm. for the first clear flour, and 48 mgm. for the second clear flour. The increases in diastatic activity due to ether extraction of the same flours were 35, 61, and 111 Rumsey units, respectively. As changes in H-ion concentration due to extraction were insufficient to explain the changes in diastatic activity, it is believed that the extraction with ether reduced the size of particles in the flour starch, thereby rendering it more readily hydrolyzable by the diastatic enzymes.

As the materials extracted from flour with ether consist, for the most part, of the true glycerides of the fatty acids, it was thought that these could be replaced by the addition of a commercial fat and certain conclusions could be drawn as to whether the improvement effected by ether-extraction was due to removal of true fats or to the removal of other ether-soluble substances. It was found that the addition of even large quantities of lard to the extracted flour did not reduce the color and texture of the bread baked from it to the same degree as in the flour improved by ether extraction, hence it was concluded that materials other than true fatty acid glycerides were responsible for the poorer quality of the bread baked from natural flour as compared with that of the bread baked from ether-extracted flour. The researches of Working (1924) indicate that the phosphatides occurring naturally in wheat or flour are the specific lipoids, the presence of which in flour is responsible for the production of bread of poor quality.

Summary and Conclusions

Bread of better color, texture, and volume was produced from ether-extracted flour than from natural flour.

Low-grade flours were improved more in volume of bread baked from them than were patent flours.

Extraction of flours with ether did not affect the absorption, the wet or dry gluten, or the viscosity of the water-extracted acidulated suspension. The extensibility of ether-extracted flours was slightly lower than that of the corresponding natural flours.

Diastatic activity and reducing sugar content were higher in ether-extracted flours than in the corresponding natural flours.

Treatment of the flour used in this study with 70% alcohol or with 96% alcohol markedly lowered its quality for bread making.

Such other properties as viscosity and extensibility were also reduced. Treatment with 70% alcohol was the more injurious.

Treatment of the flour with water did not notably affect its properties for breadmaking, but its extensibility and viscosity were reduced.

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YEAST FERMENTATION IN FLOUR-WATER SUSPENSIONS

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(Received for publication February 29, 1928)

Introduction

Several methods have been used to measure the activity of yeast during fermentation. Work has been done on this problem by Alway and Hartzell (1909), Fulmer, Nelson, and Sherwood (1921), Johnson and Bailey (1924), and Richards (1925); but none of the workers used methods which show readily the changes in rate of fermentation as yeast growth progresses. We think that the rate of gas production as a function of the time of fermentation is important.

Method

Using the method of Kusserow to indicate the carbon dioxide given off, the device shown in Figure 1 was constructed to record the rate automatically. For two reasons the flour-water suspension was used rather than a dough: (1) the dough would be hard to handle in the fermentation bottle; and (2) the deciding factor, the freedom with which gas is released from a suspension, making the result practically independent of the gluten quality. The usual formula was 200 gm. flour, 500 cc. distilled water, and 7 gm. each of yeast, sugar, and salt. For each set of experiments yeast from the same lot was used, the experiments being run off as rapidly as possible.

The flour-water suspensions were carefully made up so that their temperature was 27° C.; the entire apparatus was operated in a proof box, automatically controlled to this temperature. As soon as the mixture was made up it was placed in the fermentation bottle of the apparatus and the clock that turns the recording cylinder was started. The water supply bottle was filled with water. A thin layer of gasoline on top prevented absorption of the gas. As carbon dioxide was given off, water was forced from the supply bottle to the rate-measuring device. Here it entered a small glass cylinder with a capillary tube opening at the bottom. As the water level rose in the glass cylinder, the piano wire spring lifted the hard rubber rod, partially floating in the water, causing the pen to record the rate of flow of the water to the device.

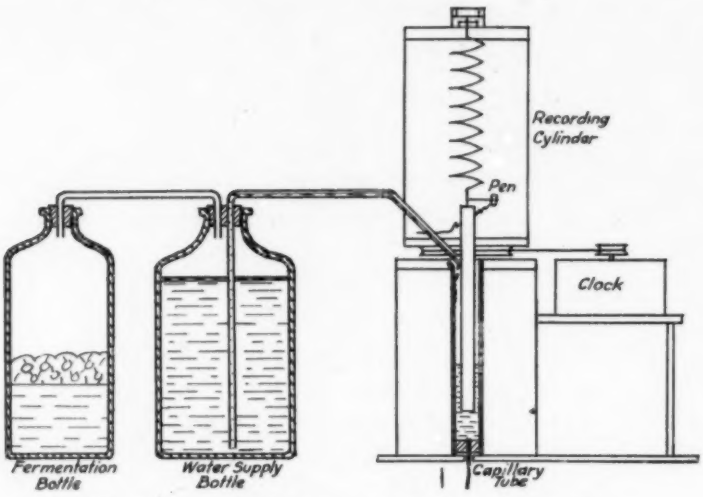

Fig. 1. Apparatus for Measuring Rate of Fermentation

As the recording device showed the fermentation rate in units which were not linear functions of the height of the curve, it was thought best to calibrate the apparatus and transfer the curves to read in cubic centimeters of carbon dioxide per minute. Figure 2 shows the calibration curve obtained by measuring the flow of water, while the device steadily recorded a certain reading. A steady flow of water for this purpose was obtained by overflowing a burette, the stop-cock of which was adjusted to give the desired flow.

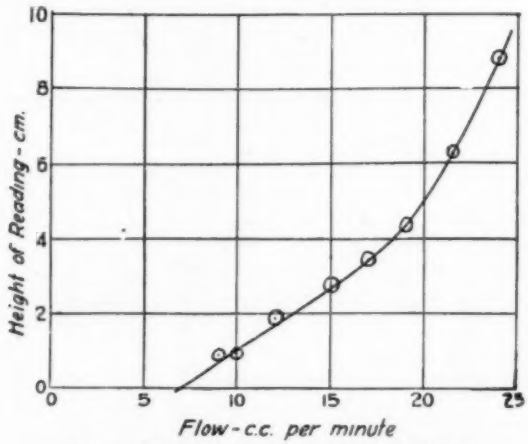

Fig. 2. Calibration Curve of Flow of Water

It was thought best to have salt and sugar in the fermentation, as these are used in baking and, as will be shown later, salt,

while it can hardly be considered a yeast food, has a very marked effect on fermentation. We could not duplicate the ratios in bread dough of salt and sugar to both flour and water, and therefore decided on the compromise formula which has already been given.

Samples of Flour Used

Eight samples of flour are referred to in the article. Their analyses are shown in Table I. These samples were all from 1927 wheat and milled on a commercial mill. All samples were bleached except E. Sample A was a pastry flour; sample B, a patent from Washington hard white wheat entirely. Samples C and D were short and long patents, respectively, from Montana hard red spring wheat. Sample E was a short clear from a blended flour of hard red spring and hard white wheat. Samples F, G, and H were clear flours from Montana hard red spring wheat.

TABLE I
ANALYSIS OF FLOUR SAMPLES

Sample	Ash	Protein	Absorption	Loaf volume	Texture
	%	%	%	cc.	
A	0.45	7.5	55	1750	fair
B	0.40	10.2	56	2340	excellent
C	0.37	11.2	60	2410	very good
D	0.43	12.0	61	2380	" "
E	0.90	13.8	60	2130	open
F	0.68	12.8	61	2320	very good
G	0.95	13.4	62	2280	" "
H	1.05	14.3	62	2260	good

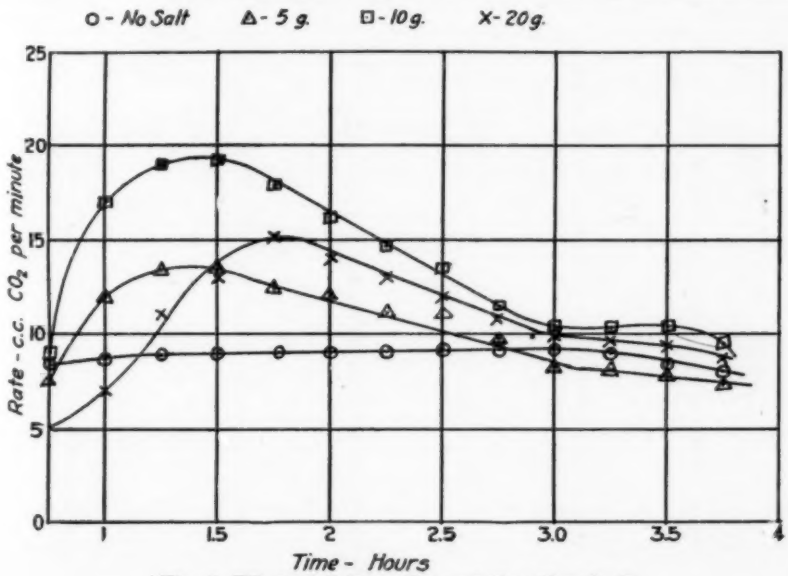

Fig. 3. Effect of Salt on Fermentation, Sample B

Effect of Salt

Figure 3 shows graphically the effect on fermentation of varying amounts of salt. Data for sample B have been used to illustrate this effect. It will be seen from this figure that the best fermentation occurred when 10 gm. of salt was used and even 20 gm. did not cause the rate of carbon dioxide production to be as low as was the case with no salt.

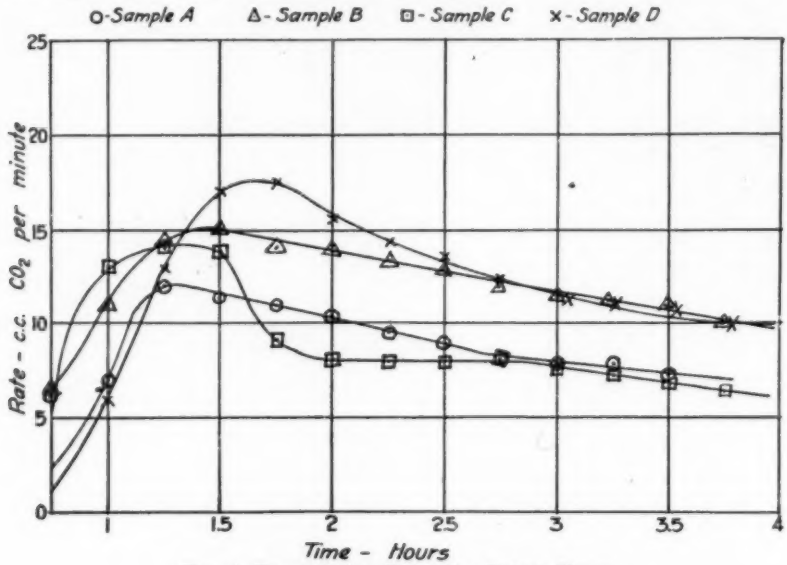

Fig. 4. Fermentation Rates for Patent Flours

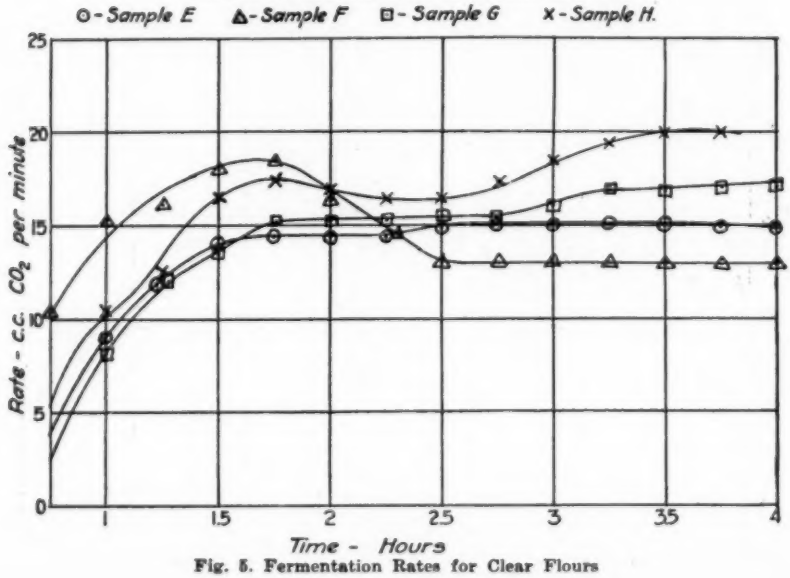

Fig. 5. Fermentation Rates for Clear Flours

Fermentation Rate of Various Flours

Figures 4 and 5 give graphical comparisons of the fermentations of all the samples of flour used for this paper. The fermentation rate of the sample of hard white flour was in general equal to that of the flour from Montana hard red spring wheat, while that of the pastry flour was somewhat less than the other flours. The clear flours, in general, had a higher rate of fermentation than the patents, particularly toward the end of the 4-hour period. This is in accord with the findings of Alway and Hartzell (1909).

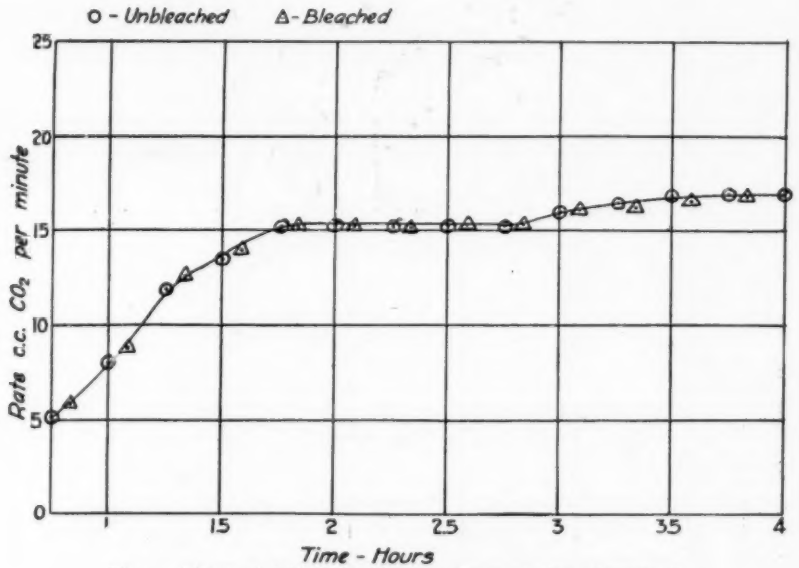

Fig. 6. Effect of NCl_3 Bleaching on Fermentation, Sample G

Effect of Oxidizing Agents

Figure 6 shows a curve of the fermentation for sample G. It will be noted that there are two sets of points on the curve, one for the unbleached flour and the other for the same flour taken after passing through the bleaching agitator. A heavy bleaching was used on the flour, 10 gm. of nitrogen trichloride per barrel, yet there was no perceptible effect on the fermentation. The oxidizing agents, sodium persulphate, bromate, dichromate, and permanganate of potassium, were tried to the extent of 10 mg. to an experiment. In no case was the rate of fermentation improved, and where potassium permanganate was added there was a marked decrease in rate.

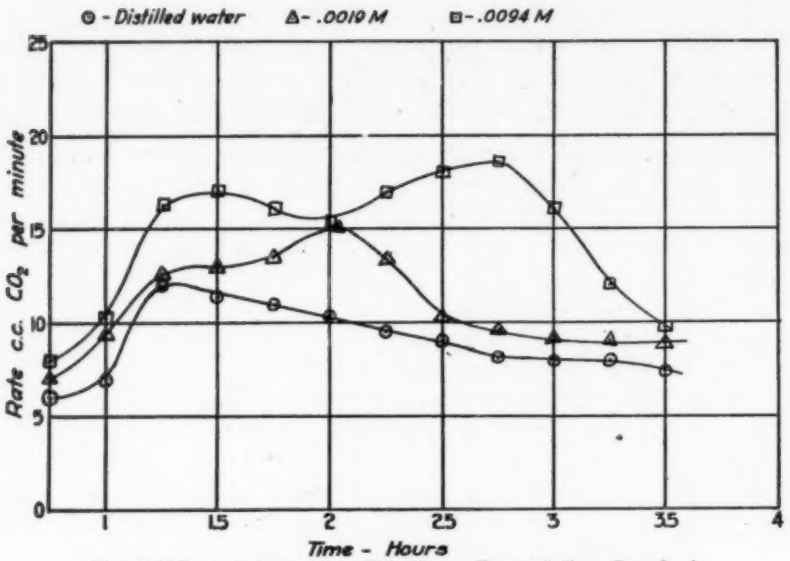

Fig. 7. Effect of Ammonium Chloride on Fermentation, Sample A

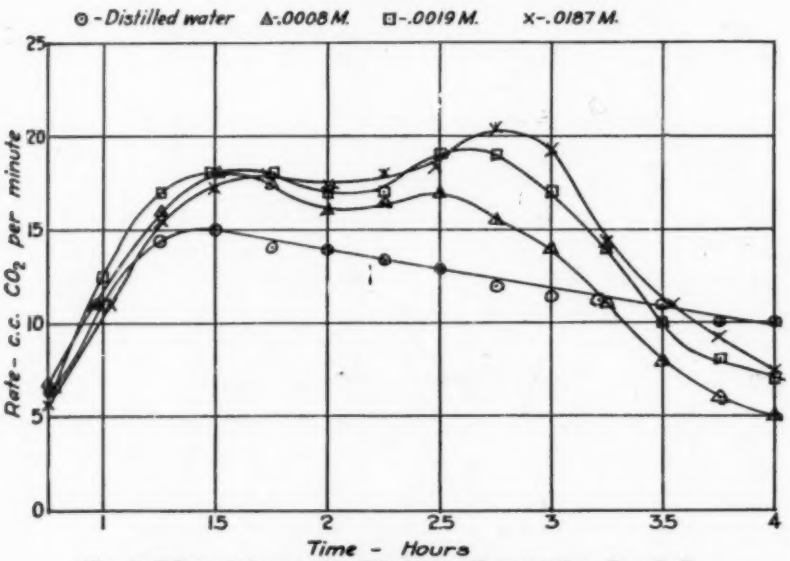

Fig. 8. Effect of Ammonium Chloride on Fermentation, Sample B

Effect of Ammonium Chloride

Figures 7, 8, 9, and 10 show the effect of ammonium chloride in varying amounts on the fermentation of samples A, B, C, and E. The fermentation rate was in all cases considerably increased. In the hard white and Montana hard red spring wheat samples a 0.002 molar solution was enough to produce practically maxi-

mum results. For the pastry sample, about 0.005 molar was needed, and for the clear 0.01 molar. Amounts up to 0.03 molar were used without appreciably reducing the fermentation. As a

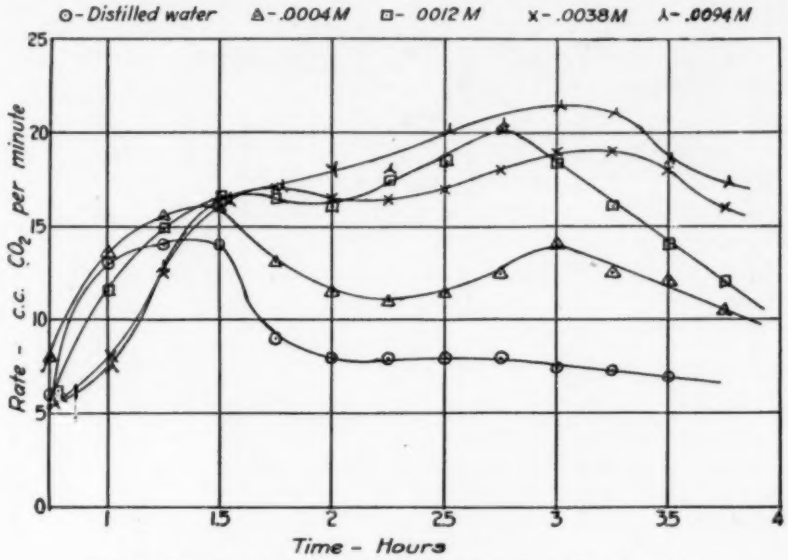

Fig. 9. Effect of Ammonium Chloride on Fermentation, Sample C

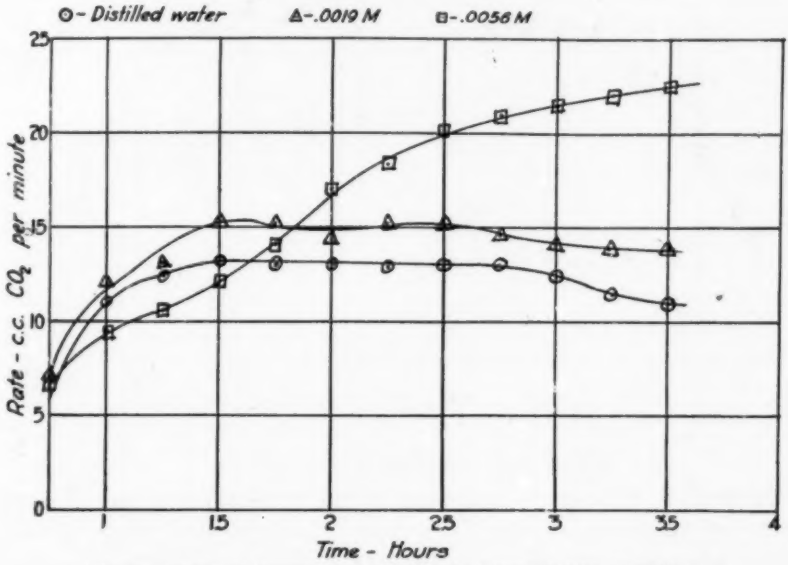

Fig. 10. Effect of Ammonium Chloride on Fermentation, Sample E

general rule the fermentation rate was somewhat decreased at the beginning of the period by ammonium chloride, but the most

marked feature was the great increase in rate toward the end of the fermentation period. However, we found that after reaching the maximum rate, fermentation with ammonium chloride decreased very rapidly and after four hours usually became less than when no ammonium chloride was used.

Effect of Alkalinity

The effect of alkalinity was not very thoroly investigated, as we thought that it was not of particular importance. Figure 11 shows what the results were in the case of sample B. When the water used for the experiment was made 0.006 normal with sodium hydroxide, only a slight decrease in the rate occurred. When the concentration of sodium hydroxide was increased to 0.012 N there was a very great decrease in the fermentation rate during the first part of the period.

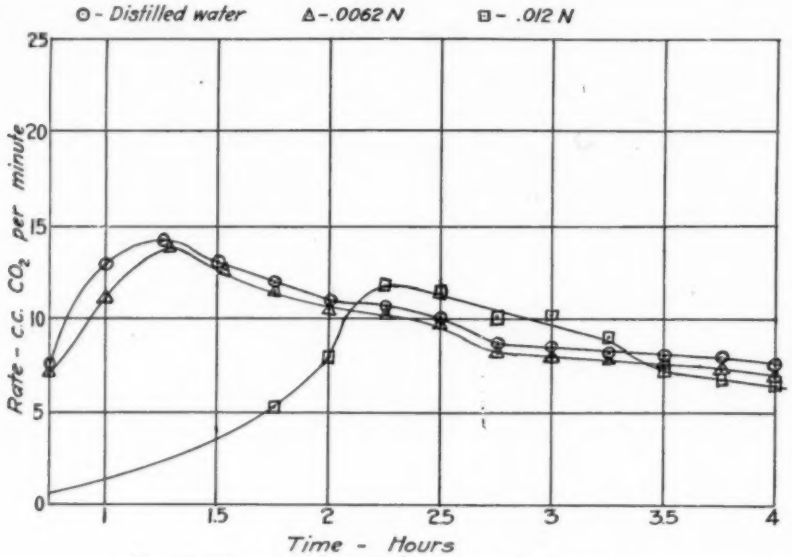

Fig. 11. Effect of Alkalinity on Fermentation, Sample B

Effect of Acidity

Figures 12, 13, 14, and 15 show the effect of increased acidity upon fermentation. There was considerable difference in the way increased acidity acted upon different kinds of flour. In sample A, the pastry flour, only a slight increase in rate was found, the maximum rate being reached when 0.0125 N hydrochloric acid was used to make up the suspension. Sample B, the hard white

patent flour, was very sensitive to increasing acidity and reached its maximum rate of fermentation when 0.003 N hydrochloric acid was used. The Montana hard red spring wheat patent flour,

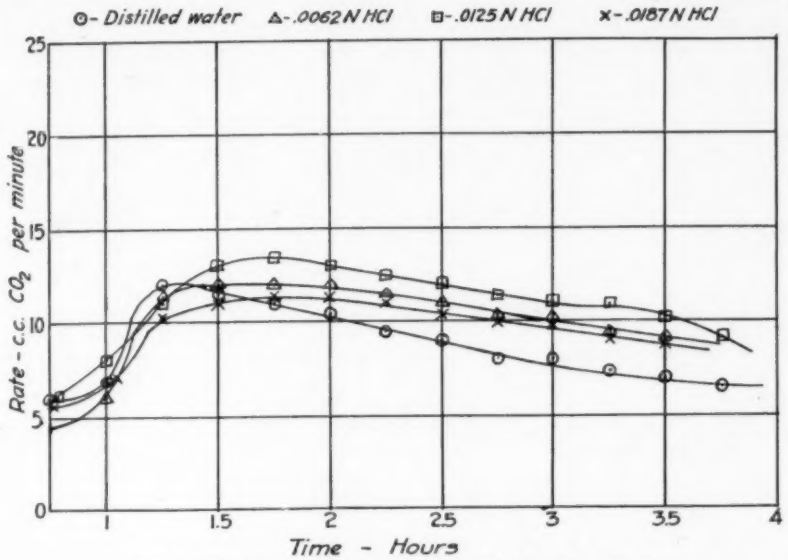

Fig. 12. Effect of Alkalinity on Fermentation, Sample A

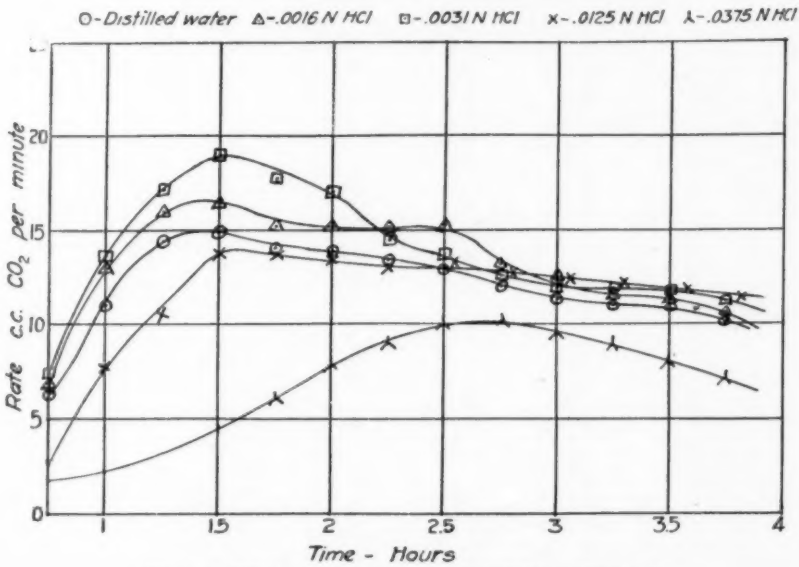

Fig. 13. Effect of Acidity on Fermentation, Sample B

sample C, did not reach its maximum fermentation rate until 0.019 N acid was used. For the clear, sample E, we cannot give the

maximum rate, as the rate still increased up to 0.037 N acid, the highest concentration used. In the other samples the rate fell off decidedly after going beyond the maximum normality with the acid solution.

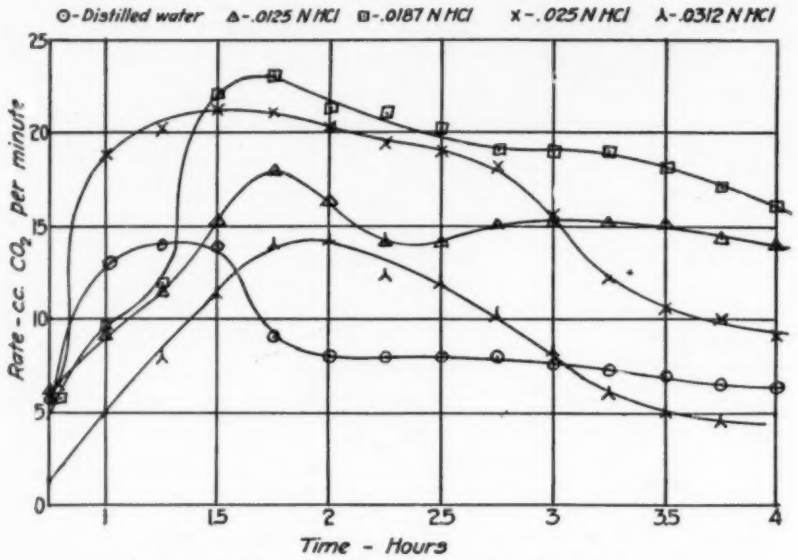

Fig. 14. Effect of Acidity on Fermentation, Sample C

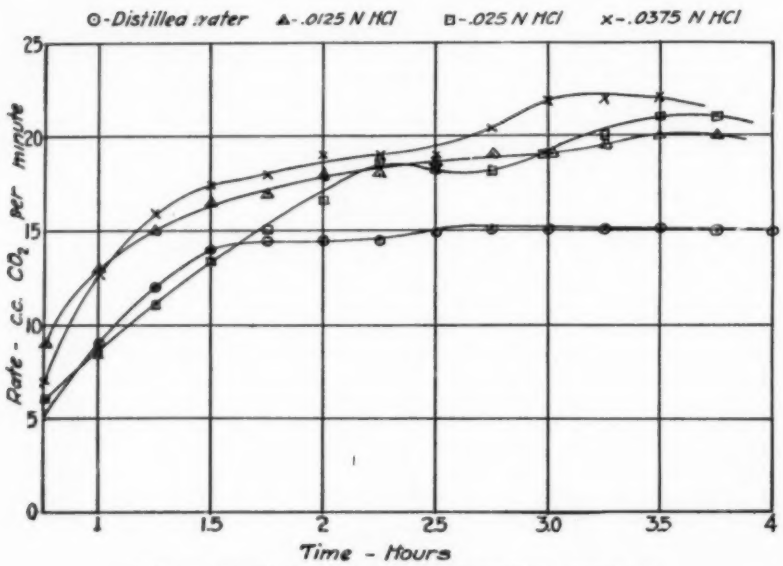

Fig. 15. Effect of Acidity on Fermentation, Sample E

Summary

A method has been developed for the convenient measurement of rate of fermentation.

The rate of fermentation has been found to be greater for clear flours than for patents.

Oxidizing agents were found to be of no value to fermentation.

Ammonium chloride greatly stimulated the growth of yeast toward the end of the fermentation period.

Alkalinity decreased yeast growth and added acidity increased fermentation. The effect of acidity varied greatly with the type of flour.

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A STUDY OF "ROPE" IN BREAD

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(Received for publication, March 20, 1928)

Introduction and Review of Earlier Work

"Rope" is a disease of bread of very common occurrence, and its symptoms are probably familiar to all millers and bakers. It is more common in hot weather than in cold and does not usually become evident in bread until at least twelve hours after the loaves leave the oven. The disease first appears as brownish spots or patches in the crumb, accompanied by a faint sickly smell. The disease spreads throughout the loaf with extreme rapidity, the whole of the crumb becoming, in the course of a day, moist and sticky. The crumb may be drawn out into long threads, a character that originated the name "rope." The smell of ropy bread is particularly objectionable and resembles that of a mixture of rotting fruit and bad cheese; it has also been described as similar to that of decomposing over-ripe melons.

Many investigations have been carried out on the problem and all workers are agreed that the disease is due to the activities of some form or forms of *Bacillus mesentericus,* which belong to the group of slime-forming organisms. The slime-forming bacteria are of very wide occurrence. They are found in the soil, from which they find their way to potatoes (the so-called potato bacteria), on grain (and hence into flour, into bakeries, and into beer), on cattle (and hence into milk), on fruit (and hence into wines). Under proper conditions of temperature, moisture, and acidity they develop rapidly and make the wine, beer, or wort (loaf) so thick and viscous that it can be drawn out into threads, hence the name "rope" is given to this type of disease in whatever substance it may appear. The action is due to the formation of a gum and a sugar (probably mannite) through the decomposition of carbohydrates such as starch by the enzyme amylase in which the rope organisms appear to be particularly rich.

To the specific name of the organism the word *viscosus* is sometimes added and some other term to denote whether the bread, milk, or wine, etc., organism is intended. The organism producing rope in bread is sometimes called *Bacillus mesentericus panis viscosus.* Much biological research has been done on this problem and it is considered probable that rope in bread is not always brought about by one and the same organism, but that several kinds are or may be involved. Lloyd and McCrea (1918) isolated and studied six strains of bacilli of the

mesentericus group, each of which was capable of producing rope in sterilised bread. All, however, are closely related and very similar, and in the absence of precise knowledge it is sufficiently accurate for practical purposes to use the group name *B. mesentericus* for these organisms, remembering that we are dealing, probably, with a group of closely related organisms rather than with a particular variety. The organisms can readily be separated from ropy bread and observed under the microscope in the following way. A little of the sticky material is removed from the loaf by means of a wire, smeared over a microscope slide and dried by keeping in a warm place, e.g., over a hot-water radiator. When quite dry the smear should be moistened with a 5% solution of methylene blue in water and then quickly washed under the tap, using a gentle stream of water. Such organisms are stained deep blue and are easily visible in the microscope as dark blue rods, usually separate, rarely as short chains.

Such organisms were first discovered in ropy wine, beer, and wort by Pasteur, and were studied in more detail by E. Laurent in 1885.

Laurent (1885) studied the organisms in ropy bread and isolated similar organisms from flour, wheat, maize, rye, and soil. He made the further observations that the *spores* of these organisms present in the loaf were not killed by baking and that the bacilli will not develop in bread above a certain acidity. Laurent was the first to recommend the use of vinegar in the control of rope.

Vogel (1897) confirmed Laurent's observation that acid breads were rarely infected. He further investigated the influence of moisture, temperature, and physical condition of the bread on the development of rope.

Watkins (1906) showed that the disease will not develop in bread if the temperature is kept below 65° F., but will develop rapidly, if the organisms are present, above 77°. This was confirmed by Lloyd and McCrea (1918).

The most favourable temperature appears to be about 100° F.

The high resistance shown by the spores of the rope organisms to the temperature of baking was established by Watkins (1906) and was examined more carefully by Lloyd and McCrea (1918). The latter workers showed that five of the six strains of rope organisms examined by them produced spores that could resist the temperature of boiling water for periods of from 30 minutes to 6 hours. As the interior of a loaf rarely, if ever, exceeds this temperature, it is obvious that a more or less prolonged baking time or higher baking temperature will not seriously interfere with the ability of the spores, if present,

to develop subsequently when conditions are favourable. At the same time, in a well baked-out loaf the development of rope may be retarded on account of its lower moisture content; rope will not develop at all on dry stale bread.

Use of Acids in the Control of "Rope"

As regards the effects of acidity, it is well known in the baking industry that rope is less liable to develop in bread of high acidity than in that of low acidity; in fruit cake it rarely develops on account of the high acidity caused by the currants and other fruits used. Watkins (1906) states that 0.3 to 0.7 lb. acetic acid per sack will inhibit rope. The minimum amount advised was 0.3 lb. while more than 0.7 lb. injured the gluten and produced bread of poor quality. Lactic acid is uncertain in its action below 0.6 lb. per sack (Watkins); on the other hand, some investigators state that 0.7 lb. per sack may spoil bread for commercial purposes while others say that as much as 1.4 lb. per sack may be used with favorable effects on volume. Actually the *amount* of acid added is probably of indirect importance. The really important factor is the hydrogen-ion concentration, or pH value, of the loaf. Lloyd and McCrea (1918) have shown that the development of the organisms is inhibited at a pH of 5.5 to 5.0, while the experiments of Cohn, Wolbach, Henderson, and Cathcart (1918), in America, indicated that a pH of less than 5 is necessary for rope prevention. Unfortunately this is very close to the minimum pH, below which really good bread cannot be made. Jessen-Hansen (1910) maintained that pH 5.0 was the concentration at which the best bread is made. It has been shown in the writers' laboratories (Fisher and Halton, 1928), however, that this is incorrect; the H-ion concentration of flour and dough, provided it is on the acid side of neutrality, is comparatively unimportant down to pH 5.0, below which, at a value varying with different flours, dough and loaf fall off in quality fairly rapidly. It appears, therefore, that the margin of safety, as regards pH, between immunity from rope and acid damage to dough and loaf is narrow.

Morison and Collatz (1921) also studied the effect of acids and acid salts on rope and a similar study has recently been made in the writers' laboratories.

Morison and Collatz measured pH colorimetrically, using methyl red as an indicator; our measurements were carried out electrometrically, using the quinhydrone electrode.

Some preliminary experiments were carried out on the use of acetic and lactic acids in "rope" control.

A sample of best white vinegar as sold by grocers was found to contain approximately 5% of anhydrous acetic acid. Loaves were made from doughs containing (a) no vinegar, (b) 1 pint and (c) 3 pints of vinegar per sack of flour. (A sack of flour=280 lb.) The pH of the resultant loaves was as follows: (a) 5.8; (b) 5.58; (c) 5.44. Further measurements showed that 8 pints per sack of this vinegar would be necessary to bring the bread to a pH of 5.0. With this amount the bread did not suffer in quality, but the smell of the acid in the loaf was distinct.

Morison and Collatz (1921) using a patent and a clear flour of somewhat higher natural acidity than that used by the writers, found 1½ lb. per sack of 60% acetic acid was required to inhibit rope; with this amount the bread from the patent flour was of poor quality (pH = 4.8 or less) while that from the clear was excellent (pH = 4.9 and 5.0). With ¾ lb. per sack the patent flour produced bread of excellent quality (pH = 5.2), but rope developed in about 5 days.

Our determinations showed that 1½ lb. per sack of 50% lactic acid would be necessary to bring the pH of the *loaf* to 5.0±.

Morison and Collatz (1921) found that the above quantity prevented rope with the patent flour (pH of loaf, 4.9) and delayed it for 4 days with the clear flour (pH of loaf = 5.2); the loaf from the patent flour was of inferior quality, that from the clear was good.

Morison and Collatz also studied the effects of hydrochloric acid, sulphuric acid, acid sodium sulphate, and mixtures of acid sodium sulphate ($NaHSO_4$) and acid potassium phosphate (KH_2PO_4). None of these were successful in preventing rope without at the same time producing poor loaf quality.

Experiments with Tartaric Acid

Loaves were baked from various flours to which varying amounts of tartaric acid had been added, and it was found that with patent flours 6 oz. per sack, and with straight-run flours 8 oz. per sack, would bring the bread to a pH of approximately 5, the exact value depending on the original pH and buffer value of the flour.

Several loaves were baked from different flours to which tartaric acid had been added. No detrimental effects were observed on dough or loaf quality, while in certain cases an improvement was noticed.

Experiments were conducted using short and long (e.g., 12 hours) baking processes and in neither case were the loaves adversely affected (see also Fisher and Halton, 1928). Since this acid had no ill effects on the bread, it was thought to be a suitable substance to study in connection with the control of rope.

Four series of four loaves each were made up, using our usual routine baking formula of 2% yeast, 1¼% salt, and 3¼ hours fermentation time (including proving) at 80° F. The liquor used in making the loaves was heavily inoculated with a water suspension of ropy bread. The flour used was a patent. For the second, third, and fourth series of loaves, tartaric acid was added to the liquor in the proportions of 4, 5, and 6 oz. per sack of flour, respectively. There were, therefore, four loaves containing no acid, four with 4 oz. per sack, four with 5 oz. per sack, and four with 6 oz. per sack. The loaves were left on the laboratory bench over night and the next day one from each series was cut and its pH and buffer value were determined. The uncut loaves were then placed in an incubator maintained at 80° F. to keep them under temperature conditions favorable to the rope organisms. When the loaves had been in the incubator two days, a second of each series was cut, examined for rope, and its pH and buffer value determined. The third day a third of each series was cut and examined and the remaining four loaves were cut when they had been in the incubator five days. The loaves containing no acid became badly ropy within two days. The loaf, however, which contained 4 oz. per sack of tartaric acid was found to have only a faint smell and stickiness after being kept at 80° F. for two days, and it was not until the third day that it was badly ropy. The loaf containing 5 oz. per sack was perfectly sound on the second day, but on the third day rope began to develop. The loaf containing 6 oz. per sack was quite sound on the third day and rope did not become evident in it until the fifth day in the incubator.

Thus a strong infection of rope was delayed for about one day by 4 oz. per sack, for two days by 5 oz. and for three days by 6 oz. per sack.

Effect of "Rope" Development on Hydrogen-Ion Concentration and Buffer Value of Bread

The effect of development of rope on the pH of this bread is interesting, and is shown in Table I.

TABLE I

Effect of "Rope" Development on pH of Bread (Patent Flour)

Age of loaf	pH of Loaf			
	1 Day	3 Days	4 Days	6 Days
Series I, no acid	5.69 (7)*	6.33 (11)†	6.62 (12)†	6.97 (15)†
" II, 4 oz. per sack	5.15 (4)	5.88 (9)‡	5.91 (10)†	6.93 (14)†
" III, 5 " " "	4.99 (3)	5.28 (5)	5.73 (8)‡	7.00 (16)†
" IV, 6 " " "	4.86 (1)	4.91 (2)	5.36 (6)	6.62 (13)†

*The numbers in brackets indicate the positions of these loaves in Fig. 1.

†Badly ropy. ‡Very slightly ropy.

With the development of rope the bread becomes progressively less acid. Even when acid has been previously added to the flour to bring the pH as low as 5 or lower, the acidity gradually disappears. As in Series III, Table I, the H-ion concentration may become after five days only one-hundredth its value at the start.

The buffer values of these loaves also show big changes; the figures are given in Table II.

TABLE II

EFFECT OF "ROPE" DEVELOPMENT ON BUFFER VALUE OF BREAD (PATENT FLOUR)

Age of loaf	pH of Loaf			
	1 Day	3 Days	4 Days	6 Days
Series I, no acid	0.88 (7) *	0.92 (11)†	0.72 (12)†	0.50 (15)†
" II, 4 oz. per sack	0.66 (4)	1.00 (9)‡	1.02 (10)†	0.48 (14)†
" III, 5 " " "	0.59 (3)	0.70 (5)	0.98 (8)‡	0.42 (16)†
" IV, 6 " " "	0.52 (1)	0.52 (2)	0.81 (6)	0.72 (13)†

*The numbers in brackets indicate the positions of these loaves given in Fig. 1.
†Badly ropy.
‡Very slight ropy.

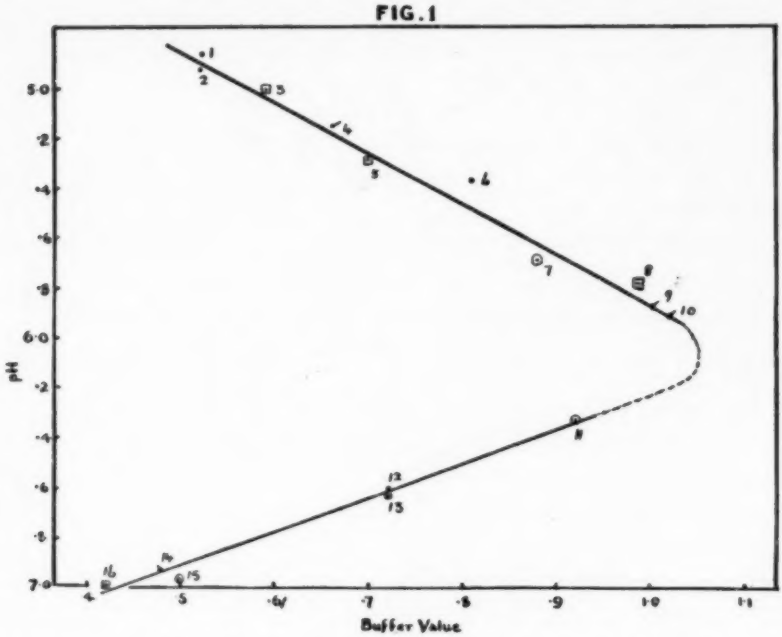
The writers adopt the following technique in measuring the buffering action of bread: The loaf is cut in two, and 100 gm. of the crumb from one half is well mixed with 500 cc. of water and the mixture is occasionally stirred. At the end of half an hour it is allowed to settle and the pH of the supernatant liquid is measured; 100 gm. of the crumb from the other half of the loaf is well mixed with 100 cc. of N/100 lactic acid and 400 cc. of water. After occasional stirrings during half an hour the mixture is allowed to settle and the pH of the supernatant liquid is measured. The difference between the two pH readings is taken as the buffer value of the loaf; this value is obviously numerically smaller, the bigger the buffering effect.

The buffering effect of the bread decreased, i. e., the buffer value became numerically greater, up to the time the rope appeared to be very fully developed, after which the buffering effect increased.

The results given for the 16 loaves in Tables I and II are shown graphically in Figure 1, in which loaf pH is plotted against buffer value. The diagram shows the alterations in pH and buffer value as rope developed and brings out a number of interesting points. In the earlier stages of rope development both H-ion concentration and buffering effect decreased; from pH 6.0-6.2 (with this particular flour; with other flours the change might occur at different pH) on, the H-ion concentration continued to decrease, although more slowly (as indicated by the smaller slope of the lower half of the curve) while the buffering effect steadily increased. (It is this increase in buffering

effect that is the cause of the slowing up of the change in H-ion concentration.) The first seven loaves indicated on the upper part of the curve showed no obvious signs of rope; loaves 8 and 9 were slightly, but distinctly ropy; the last seven, i. e., those on the lower curve, were

FIG. 1



very ropy. Although loaves 3 to 7 showed no obvious signs (e.g., smell and stickiness) of rope, the organisms must have been very active in these loaves as indicated by the rapid change in pH and buffer value. (Healthy loaves do not change in pH and buffer value in this way on keeping; e.g., with another sample of the same patent flour containing 6 oz. of tartaric acid per sack, the pH and buffer values of the healthy loaves produced were as follows:

Age of loaf (days)	1	2	3	4
pH	4.87	4.85	4.85	4.86
Buffer values	0.53	0.54	0.51	0.51)

Why, then, was no smell developed? The answer seems to be that in loaves 1 to 7 there was sufficient sugar, soluble nitrogen, and soluble phosphate to keep the organisms supplied with food; hence no attack was made on loaf proteins or carbohydrates and no smell or gumminess was produced; the removal of the soluble phosphate by the organisms resulted in the observed decrease in buffering effect. After a period, the length of which depends on the acidity and soluble phosphate content of the loaf, the soluble sugar and nitrogen become

exhausted and the organisms have to attack loaf proteins for food. This decomposition of proteins is accompanied by the characteristic ropy smell; a liberation of phosphate and other buffering materials from combination occurs which converts a decreasing into an increasing buffer effect, while the ammonia and perhaps other bases formed continue to depress the H-ion concentration. This explanation is in accord with qualitative observations of earlier workers. Thus it is known that rope organisms will grow readily on agar-agar or other carbohydrate jellies without development of smell, while on glutenous media protein decomposition occurs with liberation of ammonia and characteristic smell.

It appears, therefore, that slight stickiness and faint smell in infected bread do not mark the beginning of ropiness, but rather are characteristic of a late and acute stage of the disease; beyond this stage the progress of the disease is particularly rapid and, in fact, cannot be prevented by any practicable means.

Two further series of loaves were made from a bakers' grade flour. The first series contained no tartaric acid and the second series 8 oz. per sack. The liquor used in making the loaves was inoculated with a water suspension of ropy bread and the loaves were kept in an incubator as before. In this case rope was detected in the loaves containing no acid one day after being put in the incubator, and in the loaves containing acid, two days after. The change in pH with the development of rope is shown in Table III.

TABLE III

Effect of "Rope" Development on pH of Bread (Bakers' Grade or Clear Flour)

Time of loaves in incubator	pH of loaf			
	0 Day	1 Day	2 Days	3 Days
No acid	5.86	6.02*	6.70*	6.73*
8 oz. per sack	5.02	5.23	5.64*	6.52*

*Ropy.

It will be noticed that 6 oz. of tartaric acid per sack resulted in a loaf pH of 4.86 with the patent flour (Table I), whereas 8 oz. per sack with a more highly buffered bakers' grade produced a loaf pH of 5.02; hence rope developed more rapidly in the latter case than in the former. It is evident that although earlier biological studies have shown that rope organisms will not grow in artificial media at pH less than 5.0, they will grow in *bread* at pH apparently lower than 5.0, although their initial development is delayed. The reason for this is possibly the following: The loaf pH as measured by us is the average of the entire loaf crumb. As shown in an earlier study (Fisher and Halton, 1928), the whole of the crumb does not possess this value;

replicate determinations made on small portions of crumb were found to differ in some cases by as much as pH 0.20 to 0.25. Even in the best made loaf there will be regions of less and regions of greater acidity, small places, perhaps, of acidity sufficiently low (e.g., pH greater than 5.0) for rope organisms to begin to develop locally. Once a beginning has been made, the whole loaf will in a comparatively short time be infected, owing to the rapid production of ammonia and consequent decrease in acidity. In other words, once infection has begun, conditions become rapidly and progressively more and more favourable to the organisms.

Several other series of patent and lower grade flours, with and without the addition of tartaric acid, and heavily inoculated with rope, were baked with results entirely confirmatory of the earlier series discussed above. The results of two series, one with a patent flour and one with a bakers' grade, are given in Tables IV and V.

TABLE IV
EFFECT OF TARTARIC ACID ON "ROPE" DEVELOPMENT
(Patent Flour)

	pH of loaf				Buffer value of loaf			
Days in incubator	0	1	2	3	0	1	2	3
Tartaric acid added								
None	5.63	6.44*	6.74*	7.19*	0.73	1.07*	0.62*	0.41*
4 oz. per sack	5.19	5.20	6.04*	6.82*	0.56	0.56	0.87*	0.53*
5 " " "	5.06	5.10	5.67	6.51*	0.52	0.57	0.81	0.73*
6 " " "	5.01	5.00	5.14	5.89†	0.49	0.49	0.56	0.87†

*Badly ropy.
†Slight ropy.

TABLE V
EFFECT OF TARTARIC ACID ON "ROPE" DEVELOPMENT
(Bakers' Grade or Clear Flour)

	pH					
Days in incubator	1	2	3	4	5	7
Tartaric acid added						
None	5.76	5.98*	7.03*	6.92*	....	....
2 oz. per sack	5.48	5.91*	6.87*	6.98*	....	....
4 " " "	5.24	5.78	6.27*	6.95*	....	....
6 " " "	5.08	5.13	5.86	6.17*	6.64*	....
8 " " "	4.90	4.88	4.88	4.90	4.90	5.43*
	Buffer values					
Days in incubator	1	2	3	4	5	7
Tartaric acid added						
None	0.75	0.52*	0.34*	0.39*	.....	.....
2 oz. per sack	0.66	0.69*	0.40*	0.41*	.....	.....
4 " " "	0.60	0.83	0.56*	0.43*	.....	.....
6 " " "	0.53	0.56	0.84	0.57*	0.39*	.....
8 " " "	0.48	0.43	0.40	0.44	0.45	0.69*

*Badly ropy.

It appears that the incidence of rope may be delayed for two or three days by the addition of 4 oz. per sack of tartaric acid to a patent flour or 6 to 8 oz. per sack to a bakers' grade. The cost of such treatment, with tartaric acid at ls. ld. per pound would be 3¼ to 6½d. per sack, a very high cost for the partial protection afforded.

Experiments with Acid Calcium Phosphate

Loaves were baked from five different flours, A, B, C, D, and E, containing: (a) no added phosphate, (b) 1 lb. per sack of added phosphate. The pH of the loaves was as follows:

	No added phosphate	With added phosphate
A	6.06	5.64
B	5.95	5.58
C	6.03	5.62
D	5.95	5.55
E	5.95	5.64

Further experiments on one of these flours showed that the alteration in pH was approximately proportional to the amount of phosphate added over the range studied. The amount required to produce loaves of pH = 5.0 from the above flours would therefore be at least 2½ lb. per sack more for low grade than for patent flours.

Two series of tests were carried out with the patent flour (which was some months old and of rather high H-ion concentration) and with freshly purchased No. 2 N. Manitoba (straight run) flour, with and without added phosphate. The doughs were inoculated with rope and the loaves were kept in an incubator at 79-80° F. for a week. One loaf of each series was examined each day and its pH and buffer value were determined. The results are given in Tables VI and VII. In both series none of the loaves containing 2 lb. of acid phosphate per sack developed rope, their pH and buffer values remaining substantially unaltered for seven days. With the Manitoba flour, the loaf containing 1½ lb. phosphate per sack was ropy on the fourth day although the pH showed that the rope organisms were active on the third day. One lb. of phosphate per sack seemed to have no effect in delaying rope.

TABLE VI

ACTION OF ACID CALCIUM PHOSPHATE AS A "ROPE" PREVENTIVE ON A PATENT FLOUR

	pH					
Days in incubator	1	2	3	4	5	7
Phosphate added						
None	5.47	6.52*	6.79*	7.16*	....	....
2 lb. per sack	4.86	4.93	4.91	4.92	4.81	4.81
3 " " "	4.88	4.98	4.94	4.95	4.88	4.80
4 " " "	4.89	4.91	4.91	4.87	4.83	4.80
	Buffer values					
None	0.66	0.97*	0.48*	0.48*	....	....
2 lb. per sack	0.30	0.38	0.32	0.37	0.33	0.24
3 " " "	0.28	0.40	0.37	0.40	0.31	0.19
4 " " "	0.35	0.39	0.37	0.38	0.36	0.26

*Ropy.

TABLE VII

ACTION OF ACID CALCIUM PHOSPHATE AS A "ROPE" PREVENTIVE IN NO. 2 N. MANITOBA (STRAIGHT-RUN) FLOUR

Days in incubator	pH 1	2	3	4	5	7
Phosphate added						
None	6.46	6.72*	7.35*	.....	.....	.....
1 lb. per sack	5.68	6.29*	6.94*	.....	.....	.....
1½" " "	5.38	5.36	6.00	6.16*	6.55*	.....
2 " " "	5.19	5.19	5.18	5.12	5.14	5.01
	Buffer values					
None	0.88	0.57*	0.45*	.....	.....	.....
1 lb. per sack	0.58	0.52*	0.41*	.....	.....	.....
1½" " "	0.44	0.43	0.43	0.40*	0.30*	.....
2 " " "	0.36	0.36	0.28	0.25	0.34	0.30

*Ropy.

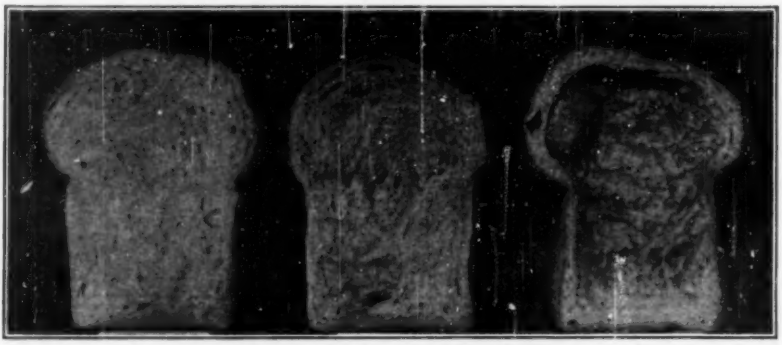


Plate I. Patent flour—2 lb. loaves—3 days at 80° F.
(1) Contains 1½ lb. of acid calcium phosphate per sack—quite fresh
(2) Contains 6 oz. of tartaric acid per sack—just ropy
(3) Control loaf—very ropy

These experiments showed that acid calcium phosphate is much more effective than tartaric acid as a preventive of rope. All the loaves treated with tartaric acid of pH between 5.0 and 4.86 developed the disease after three to six days, whereas 2 lb. of phosphate per sack appeared to prevent the disease completely, even when the loaf pH was as high as 5.19. To test this point further, other series of inoculated loaves were baked from the same patent flour without and with phosphate and without and with tartaric acid. Such amounts of phosphate and tartaric acid were added that the pH of the treated loaves was the same in both cases. Further series were baked containing acid potassium phosphate, and one series without yeast but with a self-raising mixture of 3 lb. of sodium bicarbonate and 6 lb. of acid calcium phosphate per sack. The object of the last series was to ascertain whether the preventive action of the phosphate was a specific action or whether (as with tartaric acid, but more effectively)

it was caused solely by a high H-ion concentration. If the former, self-raising flours (containing large amounts of phosphates at comparatively large pH) ought to be immune from rope; if the latter, self-raising flours ought to be sensitive to rope. The results are given in Table VIII and indicate that:

(1) Acid potassium phosphate is very much less effective as a preventive of rope than acid calcium phosphate. This is presumably due to the fact that acid potassium phosphate has less effect on the pH of flour than has acid calcium phosphate.

(2) The self-raising flour, in spite of its large phosphate content, was more sensitive to rope than the untreated flour; its pH was also greater. This suggests that the preventive action of the phosphate is not specific, but is probably a real H-ion concentration effect.

(3) Acid calcium phosphate really is much more effective than tartaric acid as a rope preventive. Thus the loaf of pH 5.16 containing tartaric acid was slightly ropy on the second day; the loaf of almost identical pH (5.17) containing phosphate (1½ lb. per sack) did not become slightly ropy until the seventh day, while the loaf with 2 lb. phosphate per sack and pH of 5.14 remained healthy for seven days.

Plate II. Similar Series to Plate I, Only 1 lb. Loaves—4 Days at 80° F.

(1) Loaf containing phosphate—quite fresh
(2) Loaf containing tartaric acid—very ropy
(3) Control loaf—very ropy

TABLE VIII

EFFECT OF ACID CALCIUM PHOSPHATE, ACID POTASSIUM PHOSPHATE, AND TARTARIC ACID ON "ROPE" (PATENT FLOUR)

	pH					
Days in incubator	0	1	2	4	7	8
Material added (per sack)						
None	.....	5.64	7.06*	.....	.....	.....
4 oz. Tartaric acid	.....	5.16	6.06†	.....	.....	.....
1½ lb. Acid calcium phosphate	.....	5.17	5.12	.....	6.04†	.....
1½ " " " "	5.20	5.19	5.17	5.36	.....	†
2 " " " "	.....	5.14	.....	.....	sound	.....
1½ " " potassium "	5.58	6.02	6.37*	.....	.....	.....
2 " " " "	5.55	5.88	6.29*	.....	.....	.....
Self-raising mixtures‡	6.48	6.47*	6.70*	.....	.....	.....
	Buffer values					
None	.....	0.85	0.63*	.....	.....	.....
4 oz. Tartaric acid	.....	0.64	0.84†	.....	.....	.....
1½ lb. Acid calcium phosphate	.....	0.44	0.50	.....	†	.....
1½ " " " "	0.42	0.39	.....	.....	.....	†
2 " " " "	.....	0.40	.....	.....	sound	.....
1½ lb. Acid potassium phosphate	0.68	0.69	*	.....	.....	.....
2 " " " "	0.67	0.70	*	.....	.....	.....
Self-raising mixtures‡	0.25	0.25	*	.....	.....	.....

*Ropy.

†Slightly ropy.

‡Sodium bicarbonate, 3 lb. per sack, Acid calcium phosphate 6 lb.

Further experiments were carried out with 1½ and 2 lb. of acid calcium phosphate per sack on a considerable number of millers' grists sent to the laboratories for baking tests. The loaves, all of which had been heavily inoculated with the disease, were kept for several days in an incubator at 80-81° F. Every loaf containing no added phosphate was badly ropy on the second day; none of the loaves containing 2 lb. phosphate per sack showed any signs of rope on the fifth day; of the loaves containing 1½ lb. phosphate per sack, all the patents were healthy on the fifth day, whereas all the straight-run and bakers' grades were more or less ropy on the fifth day.

Two further series of tests with a bakers' grade flour are recorded in Table IX. This table includes the only case we have observed of a bakers' grade developing rope in the presence of 2 lb. of acid calcium phosphate per sack. Four days were required for the rope to become evident in this sample. It is also shown that phosphoric acid is less effective as a preventive of rope than acid calcium phosphate, although it is more effective in altering H-ion concentration; thus the loaf containing phosphoric acid developed rope in six days although its pH was 4.92, while the corresponding loaf with 2 lb. acid calcium phosphate per sack and pH of 5.32 showed no signs of ropiness after six days.

TABLE IX

ACTION OF ACID CALCIUM PHOSPHATE AS A "ROPE" PREVENTIVE IN A BAKERS' GRADE OR CLEAR FLOUR

Days in incubator	pH 0	1	2	3	4	5	6
Material added (per sack)							
1st Series:							
Blank	5.81	6.01*	....	....	....	....	....
1½ lb. Acid calcium phosphate	5.41	5.32	5.66	5.96‡	6.61†	....	....
2 " " " "	5.29	5.30	5.28	5.40	5.74‡	....	....
2nd Series:							
Blank	5.58	6.13	6.09†	6.90†	....	....	....
2 lb. Acid calcium phosphate	5.32	5.34	5.32	5.31	....	5.29	5.15
2½ " " " "	5.15	5.18	5.16	5.20	....	5.12	5.10
½ lb. Phosphoric acid	4.92	4.94	4.93	4.95	....	5.14	5.56‡

*Very badly ropy.

†Ropy.

‡Slightly ropy.

Plate III. Bakers' Grade Flour—2 lb. Loaves

(1) Control loaf—very ropy after 2 days at 80° F.
(2) Loaf containing 2 lb. per sack of acid calcium phosphate—quite fresh after 2 days at 80° F.
(3) Similar to 2—quite fresh and no sign of rope after 6 days at 80° F.

It is not clear why acid calcium phosphate should be so much more effective than tartaric or phosphoric acid as a preventive of rope. It is undoubtedly largely, if not entirely, a H-ion concentration effect; possibly the addition of phosphate ensures a more uniform pH throughout the loaf so that there are fewer regions of acidity low enough for rope to develop. The facts, however, appear to be well established, as is indicated by the tables and photographs given in this paper.

Many millers use acid calcium phosphate as an improver at the rate of about 12 oz. per sack of flour. By increasing the amount to 1½ lb., at an extra cost of 1¼d. per sack, the loaves produced should remain free from rope for five or six days even in the hottest weather. By raising the amount to 2 lb., at an extra cost of 1¾d. per sack, the flour and loaves should be altogether immune from the disease.

We have been unable to detect any ill effects on dough or loaf quality due to the presence of 2 lb. per sack of acid calcium phosphate.

It is not suggested that millers should treat all their flour with these quantities of phosphate as a mere safeguard against rope. Although the disease is not uncommon in summer,[1] it is certainly not so widespread as to justify such a general treatment of the miller's whole output. But in cases of serious complaint from bakers, every separate flour should be tested for rope by the making of a loaf, as described below, and flour shown to be infected could be treated with phosphate.

Practical Conclusions

The chief practical conclusion to be drawn from this work is that acid calcium phosphate at the rate of 2 lb. per sack of flour appears to completely prevent rope development. Nevertheless, the best and cheapest method of rope prevention is a cool, well-ventilated bread store. There is little likelihood of rope developing in bread that is stored under conditions of good ventilation at a temperature below 65° F. These conditions, combined with the strictest cleanliness of troughs, bakeries, and bread stores, are the first essentials in rope prevention.

Practically all flours are liable to contain spores of the rope organism. The responsibility lies not with the miller, as many bakers appear to believe, but with Nature in allowing old cultivated soils to be a particularly good breeding place for organisms of innumerable kinds, both beneficial and harmful to humanity. Since the virtual disappearance of potatoes for ferment making, there can be little doubt that rope finds its way into the bakery in flour. Where, therefore, any serious outbreak of rope occurs, each separate delivery of flour in the bakery should be examined for the presence of the organism. A test for detecting rope spores in flour was devised by Watkins (1906) and described also by Jago (1921). It was stated that this

1 Many serious outbreaks occurred during the War Period, and the disease was given some attention by the Royal Commission on Wheat Supplies. The greater prevalence of rope in this period was probably due, primarily, to the greatly increased flour extraction insisted upon. Since the rope organisms are present in many, especially old, soils they are found on the outside of grain grown on such soils; consequently with infected wheats the greater the flour extraction and consequently the greater the percentage of branny particles, the greater the number of organisms in the flour, and the greater, therefore, the liability to the disease. It has been known for many years that low-grade flours are more liable to rope than high-grade or patent flours. Unfortunately, nothing is known concerning the distribution of rope organisms in the various soils and countries or on different wheats. It would be an interesting study to examine for the presence of rope every lot of wheat received at a mill for a year; much valuable information might be obtained in this way concerning the kinds of wheat most liable to infection. All that can be said at present on this point is that wheat grown on old, heavily manured soils, e.g., English wheat, might be expected to be more liable to infection than wheats grown on young soils such as Australian and Canadian.

Two further factors probably contributed to the greater prevalence of rope in the War Period: (1) Bread was not allowed to be sold until it was 24 hours old; (2) In order to minimise the loss of weight due to loss of moisture from the loaves, the baker stored his bread covered or under conditions of poor ventilation. If rope is present, both these factors would tend to promote the development of the disease.

test is so delicate that a positive result could be obtained from 0.02 gm. of ropy flour, while there was no fear that a genuinely sound flour would be condemned by its employment. Although the test is intended for practical millers and bakers, we have been unable to obtain positive results by means of the test even with flours known to be ropy or with flours we have inoculated heavily with rope. In any case, the test is more troublesome to carry out than is the making of a loaf. The best test is to make a loaf and keep it in an incubator or proving cabinet or other warm place for 48 hours. If no rope develops in this time the flour can be regarded as sound. Flour found to be infected could be kept separate from the rest until the winter months when rope is less liable to develop on account of the lower temperature; or it could be baked separately with the addition of acid calcium phosphate in doughing at the rate of 1½ lb. per sack for a patent flour or 2 lb. for a bakers' or low grade. If the flour already contains added phosphate as an improver, half the above quantities would probably be adequate.

Summary

"Rope" in bread is a disease due to the activities of various forms of *B. mesentericus*. It is similar in some of its effects to the disease of the same name observed in wine, beer, and wort.

Much of the earlier work on this disease is reviewed and it is shown that moisture, warmth, and a low degree of acidity are necessary for its development.

Many of the methods suggested for controlling "rope" depend on the addition of acids or acid substances to the dough whereby the H-ion concentration is raised sufficiently to inhibit rope development. In this connection a study has been made of acetic acid, lactic acid, tartaric acid, acid potassium phosphate, and acid calcium phosphate. Of these substances, acid calcium phosphate is the most effective as a preventive of rope and is least open to objection.

The changes in pH and buffer value of bread consequent on rope development have been determined, and these changes show that slight stickiness and faint smell in bread do not mark the beginning of ropiness (as was formerly thought), but are characteristic of a late and acute stage of the disease.

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SOME FACTORS INFLUENCING THE ABSORPTION IN EXPERIMENTAL BAKING[1]

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It is generally understood that the absorption of a flour is the quantity of water required to produce a dough of standard consistency, but certain explanations might throw a little light as to how such wide variations in percentage absorption are reported from time to time. In the first place, the word "standard" is rather misleading, for the test is not absolute.

A dough may appear to be just the proper stiffness in mixing but the retention power of a flour during fermentation, manipulation, and proving must be considered. Again, the point at which one person considers the dough to be of proper consistency may not exactly suit the touch of another. Long practice is necessary to determine absorption, and even then the question of individualism must always be taken into account. When absorption is reported in high figures, which are not practical in mixing up a dough in the bakeshop, the test has probably been run using flour and water alone. (Dedrick, 1924, Siebel, 1924.)

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In this connection, experiments were carried on with the following problems in mind:

To ascertain whether percentage absorption remained constant irrespective of the quantity of flour used.

To determine the influence on absorption of the combination of common ingredients used in bread making—salt, sugar (cane), shortening (lard), and compressed yeast (Fleischmann)—as compared with a dough made with flour and water alone.

To find to what extent the separate ingredients, in varying percentages, influenced absorption.

To note any changes in retention power during fermentation.

To note the effect of minimum and maximum absorption on the finished loaf of bread.

Wherever possible the experiments were carried through to the final product bread, so that the greatest possible amount of information could be gleaned, particular attention being paid in each case to the retention power of the flour throughout the entire process of fermentation. In all baking tests the following basic formula was employed:

340.0 gm. flour
5.1 " salt (1.5%)
11.9 " sugar (3.5%)
6.8 gm. lard (2%)
13.6 " yeast (4%)

Water to make a dough of the desired consistency for each test.

The same flour was used for all experiments—a bleached second patent bakers' flour from 1926 crop Western Canada wheat. Chemical analysis of this flour is shown in Table I.

TABLE I

ANALYSIS OF FLOUR USED

	Moisture	Ash	Protein	Gluten		Quality of gluten	Absorption	Remarks
				Wet	Dry			
	%	%	%	%	%		%	
As received	12.10	0.446	12.44	40.90	13.50	Very good	58.00	Traces Cl, NO_3
Computed to 13.5% moisture base	13.50	0.439	12.24	40.25	13.28			

The absorption method employed (according to Dedrick, 1924) was as follows:

Six tests were run, using 50 gm. flour. A different amount of water was added to each, in order to determine the correct absorption. A spatula was used for mixing and the doughs were then worked into balls with the fingers. These were compared

10 minutes after mixing. In addition, the dough balls were handled and comparisons made 30 and 60 minutes after mixing. From the six tests made, in which 58%, 59%, 60%, 61%, 62%, and 63% absorption was used, the dough with 61% was the most desirable consistency.

Experiments were carried out with 61% absorption using different amounts of flour, as follows: 20, 50, 100, 150, 220, and 340 gm.; the larger doughs, 220 and 340 gm., being mixed in a Bachman mixer. The consistency of the doughs directly after mixing and on standing was to all intents and purposes the same.

In all cases, corresponding experiments were carried out, including in the doughs the proportionate amounts of salt, sugar, lard, and yeast used in bread making and, without exception, the absorption had to be decreased 3% to get the same degree of stiffness as in doughs made with flour and water alone. When this difference was definitely established, further experiments were carried on to determine to what extent each ingredient was responsible for this decrease in absorption.

Tests were made with 61% absorption, including in the doughs besides flour and water one other ingredient, the quantities used being in accordance with basic formula and a dough made with flour and water alone serving as a standard for purposes of comparison.

Salt: This dough was slacker directly after mixing than the dough with flour and water, but it became decidedly stiffer on standing.

Sugar: This dough was slacker directly after mixing than the dough with flour and water and became still slacker on standing.

Lard: This dough was slightly slacker directly after mixing than the dough with flour and water and there was little, if any, change on standing.

Yeast: This dough was stiffer at all times than the dough with flour and water.

Experimental baking tests were made, varying the percentage of the separate ingredients, to determine the influence on absorption and to note any changes in retention power throughout the entire process.

Salt: As indicated in Table II from 0% up to 4% of salt was used and, whereas the absorption directly after mixing was decreased from 59.10% for a dough with no salt to 56.50% for a dough containing 4% salt, during fermentation there was a gradual stiffening of the doughs parallel with the increasing percentage of salt used.

Sugar: From 0% up to 7% was used and, as will be seen from Table II, the absorption directly after mixing was decreased from 60.40% for a dough with no sugar to 56.10% for a dough with 7% sugar. During fermentation the doughs became slacker, especially those which required abnormally long fermentation on account of the small percentage of sugar used.

TABLE II

INFLUENCE OF SALT, SUGAR, LARD, AND YEAST ON ABSORPTION

Salt	Absorption	Sugar	Absorption	Lard	Absorption	Yeast	Absorption
%	%	%	%	%	%	%	%
0.0	59.10	0.0	60.40	0.0	58.90	0.0	57.60
0.5	58.70	0.5	60.05	0.5	58.65	2.0	57.80
1.0	58.30	1.0	59.70	1.0	58.40	3.0	57.90
1.5	58.00	1.5	59.35	1.5	58.20	4.0	58.00
2.0	57.70	2.0	59.00	2.0	58.00	5.0	58.10
2.5	57.40	2.5	58.65	2.5	57.85	6.0	58.20
3.0	57.10	3.0	58.30	3.0	57.70	7.0	58.30
4.0	56.50	3.5	58.00	4.0	57.40	...	.....
...	.....	4.0	57.70	5.0	57.20	.....	.....
...	.....	5.0	57.10	6.0	57.00	...	.....
...	.....	6.0	56.60	7.0	56.85	...	.....
...	.....	7.0	56.10	...	.....	...	.....

Lard: From 0% up to 7% was used and, as indicated in Table II, the absorption was decreased from 58.90% for a dough with no lard to 56.85% for a dough containing 7% lard. During fermentation the tendency was for the doughs to slacken a little, this being more noticeable in doughs with an increasing percentage of lard.

Yeast: From 0% up to 7% was used and wherever practicable the doughs were carried through the baking stage. As indicated in Table II, the absorption directly after mixing was increased from 57.60% for a dough with no yeast to 58.30% for a dough with 7% yeast. During fermentation there was a gradual slackening of the doughs due to the action of yeast in softening the gluten. This condition is kept in control by the use of salt in the dough, as to a certain extent these two ingredients work antagonistically. (The moisture content and the freshness of the yeast are factors which may influence results from time to time.)

Baking tests were also made using from 53% to 64% absorption to note the effect of minimum and maximum absorption during fermentation and in the baked bread. Table III gives comparative results from this test. As shown in this table the fermentation period is shortened by low and high absorption, particularly the latter. The figures on fermentation loss indicate that when absorption is abnormal, especially to the high extreme,

TABLE III

INFLUENCE OF THE MINIMUM AND MAXIMUM ABSORPTION ON THE FINISHED LOAF

Absorption	Fermentation	Fermentation loss	Oven loss	Weight of loaf	Vol. of loaf	Loaf with 53% absorption expressed as 100%			
						Yield of bread	Vol. of loaf	Color of crumb	Texture and grain
%	min.	%	%	gm.	cc.	%	%	%	%
53	134	2.07	11.40	480.5	2270	100.0	100.0	100.0	100.0 el ti*
54	135	2.09	11.49	482.8	2280	100.4	100.4	100.5	101.0 el ti
55	136	2.10	11.60	485.0	2310	100.9	101.8	101.0	101.5 el
56	136	2.11	11.68	487.4	2330	101.4	102.6	101.5	102.0 el s
57	137	2.12	11.77	489.8	2370	101.9	104.4	102.0	102.0 el s
58	137	2.12	11.89	492.0	2400	102.3	105.7	101.5	101.5 el s
59	136	2.10	12.00	494.5	2420	102.9	106.6	101.0	101.0 c
60	134	2.06	12.09	497.1	2440	103.5	107.5	100.5	99.5 c sp
61	131	2.03	12.20	499.7	2440	104.0	107.5	99.5	98.5 c sp
62	128	2.00	12.27	502.4	2430	104.6	107.0	98.5	97.0 c sp ir
63	124	1.96	12.38	505.0	2400	105.1	105.7	97.5	96.0 c sp ir
64	120	1.91	12.47	507.7	2380	105.7	104.8	96.5	95.0 c sp ir

*Abbreviations: el = elongated, sp = spherical, ti = tight, s = silky, c = coarse, ir = irregular.

the condition of the medium is unfavorable for the best action of the yeast in the doughs; and that better results are obtained when percentage absorption is nearer the normal point.

With increasing absorption the yield of bread is also increased, but this is influenced by evaporation of water during the baking, this loss being higher when more water is used in the dough. The difference in loaf volume is greater with lower absorption figures altho with excessively high absorption, and the resultant impaired quality of the loaf, the volume is also decreased.

It is evident that inferiority in both color and texture of the bread is much more pronounced when percentage absorption is high than in the loaves with absorption to the other extreme. (This agrees with findings of Sherwood and Bailey, 1927.) With low absorption, the grain of the bread is tight and even, the cells being elongated and having only a slight tendency to holes. There is a gradual improvement in the texture until the peak is reached at 57% absorption, this having a slight advantage in both color and texture over the bread with 58% absorption. These loaves have a fine open grain and a decided silkiness in texture. From this point, with the absorption increasing, the grain gradually becomes coarse, very open, and irregular, with thick cell walls, the cells being more nearly spherical in shape. The loaves with 62%, 63%, and 64% have flat tops, the gluten having been weakened by excess water.

As a rule, bakers favor high absorption in order to obtain high yield, whereas there are other qualities which should receive consideration along with this, because high absorption may lead to trouble in mechanical manipulation. Furthermore, any advantage to be gained from the higher yield may be more than offset by deterioration in the other desirable qualities in bread.

The yield of bread per barrel is often based on percentage absorption alone, but the quantities and the kind of ingredients used, the manipulation, the retention power, and the loss throughout the whole baking process are factors which may influence the yield.

Absorption is sometimes reported as computed to a 13.5% or 15% moisture basis, but this should not be done. Under normal conditions, during the aging of flour certain physico-chemical changes are taking place whereby the flour is improved and the absorption increased irrespective of the moisture content. To a certain extent moisture does affect the absorption of a flour, but it is not correspondingly altered.

Further experimental work dealing with other ingredients used in commercial baking will be reported later.

Conclusions

1. The quantity of flour used in a dough does not appreciably alter the percentage absorption.

2. There is a difference of approximately 3% between the absorption of a dough consisting of flour and water alone and that of a dough containing salt, sugar, lard, and yeast.

3. It is much more difficult to determine the end point of absorption in a dough of only flour and water.

4. The absorption of a flour is increased with the use of salt in a dough because any slackness directly after mixing is more than offset by the stiffening of the dough during fermentation, salt having a toughening effect on the gluten.

5. Sugar is largely responsible for the decreased percentage absorption of a dough containing salt, sugar, lard, and yeast as compared with a dough made of flour and water alone.

6. Lard is to a small extent responsible for reducing the percentage absorption of a dough containing salt, sugar, lard, and yeast as compared with a dough made of flour and water.

7. The absorption of a flour is slightly increased by yeast.

8. High absorption is more detrimental to the quality of bread than low absorption.

9. Information would be more reliable if percentage absorption were reported in figures which were practical in making bread, taking into consideration the retention power during fermentation and all the other factors which influence absorption.

10. Absorption should not be computed to a 13.5% or a 15% moisture basis.

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A VOLUME MEASURING DEVICE FOR SMALL LOAVES

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(Received for publication December 23, 1927)

The loaf-measuring devices available for the measurement of standard loaves (1600-2400 cc.) are not adaptable to the measurement of loaves made with 100 grams of flour and varying in volume from 400 to 500 cc.

The standard type of loaf-measuring apparatus, based on the hour-glass principle and used in most laboratories, is calibrated in 10 cc. divisions, and cannot be read with greater precision than this figure, owing to variations in the size of seed, degree of packing, and the large bore of the measuring tube.

In order that the error shall not be relatively greater in the measurement of small loaves, it was deemed necessary to be able to make duplicate readings to an accuracy of from 2 to 3 cc. A greater degree of precision than this is not feasible in apparatus of this type.

The above considerations impressed upon us the desirability of constructing a special device adapted to the measurement of small loaves.

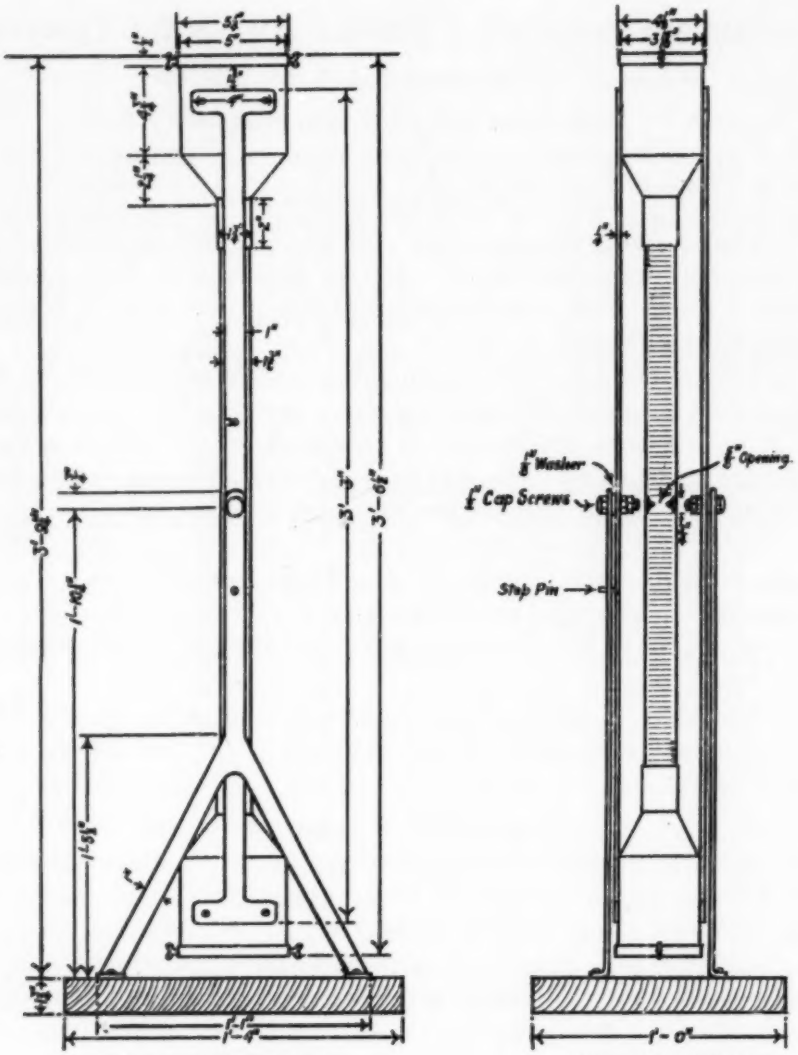
Description of Apparatus

No originality is claimed for the apparatus, which is modeled on the hour-glass type, such as manufactured by the Industrial Appliance Company, with a suitable reduction in dimensions.

An apparatus, to be built in the laboratory, must be of simple construction and easily workable materials, consequently the use of aluminum castings for the hoppers was out of the question. Sheet metal of light enough gauge to be formed to shape lacks rigidity, and it was therefore decided to build the apparatus up "piece-meal" from heavy sheet brass (3/32 inch thick).

The general construction and dimensions are illustrated in the accompanying drawing, but for the benefit of those desiring to construct a similar device, the following additional details may be helpful.

In order to obtain approximately the same capacity for each chamber, the four similar pieces, for example, the sides of the



SIDE ELEVATION. END ELEVATION.

hoppers, were first sawed out of 3/32 inch sheet brass, allowing a generous excess over the measurements. The pieces were then registered, clamped together, and filed to the correct dimensions, which are as follows:

4 sides	5 x 4¼ inches
4 ends	4¾ x 3¼ inches
4 bottom sides	5 inches wide, tapering to 1⅜ inches, with sides 2⅞ inches long

4 bottom ends .....3¾ inches wide, tapering to ⅞ inch, with sides 2⅞ inches long
2 covers or lids .....5 1/16 x 4 inches
4 sides for lids .....5¼ x ½ inch
4 ends for lids .....4 x ½ inch

Standard Loaf

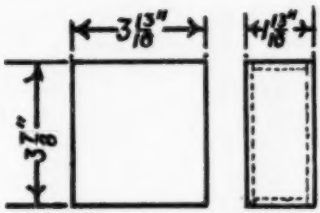
2 sides .....3 13/16 x 3⅞ inches
2 ends .....3 13/16 x 1⅝ inches
Top and bottom .....3 13/16 x 1⅝ inches

The hoppers were assembled by tinning the faces to be soldered and sweating together, thus producing a butt joint. Owing to the thickness of the brass, this method gave a very rigid and strong joint, using the minimum of solder.

In order to provide a simple means for attaching the measuring tube to the base of the hoppers and allow equalization of their capacities, the following method was adopted:

A strip of thin sheet copper 2½ inches wide was formed into a tube which fitted snugly into the measuring tube, the upper half inch being slit vertically at frequent intervals. The rectangular opening at the base of the hopper, which is approximately 1⅛x⅞ inches, was enlarged with a file to admit the copper tube which was then inserted, flanged over to the interior faces of the hopper, and smoothly soldered. The outer collar illustrated in the diagram was similarly made and fitted to the outside of the hopper.

This is the simplest method of attaching a circular tube to a rectangular opening, and the use of the internal sleeve prevents the seed lodging on the ends of the measuring tube.



The lids were assembled in a similar manner. Holes were drilled in the center of the ends of the hoppers ¼ inch from the top, bolts were fitted through from the inside and soldered in place. The ends of the lids were then drilled and slotted to correspond, and held in place by wing nuts.

One lid was fitted with two small spring brass clips to hold the loaf, while the other was drilled with a single hole, through which passes a bolt holding the standard loaf.

The standard loaf is merely a rectangular brass box, the volume of which can be adjusted to 450 cc. either by adding solder, or by filing. If the dimensions given are strictly followed, the final capacity should be very nearly 450 cc. (The volume of the standard loaf was determined by weighing the water displaced by the loaf.)

The supporting frame was constructed of 1x¼ inch strap iron, as shown in the diagram.

The measuring tube used was a standard wall Pyrex tube, 1⅜ inches outside diameter and approximately 26 inches long, in the center of which was cemented an internal brass bi-concave collar, with a ⅝ inch opening. This was turned in the lathe to a very free sliding fit, and several grooves were cut in the periphery. The grooves were filled with De Khotinsky cement, the collar was slid into place and fixed in position by rotating the tube over a flame.

Calibration of Measuring Tube

In order to have graduations spaced evenly on each side of the central collar, the following procedure was adopted:

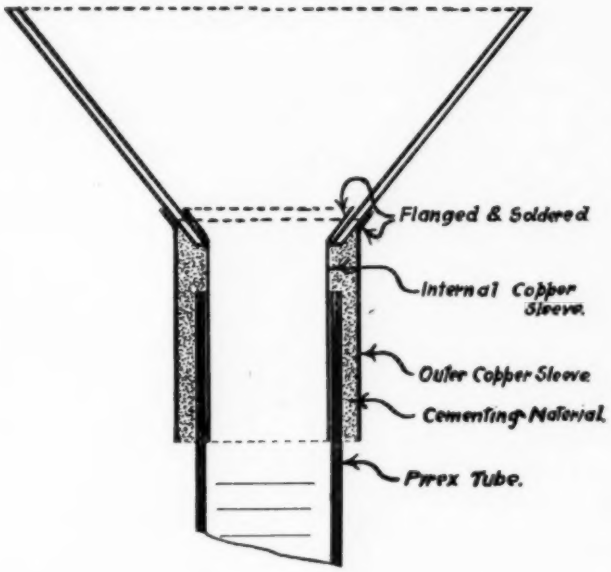
The tube was securely stoppered at one end, clamped vertically, and filled with water to the center of the internal collar, and 200 cc. water was then added. (The amount of water added should not occupy more than 11½ inches measured from the center of the tube.) An additional cc. of water was added, and the zero mark was established at this point. This addition was made to offset the error involved in inverting the meniscus in the next stage of the calibration.

The tube was then emptied, inverted, stoppered at the other end, and filled to the zero point and calibrated by the addition of 10 cc. portions of water, using a standardized pipette. This method of calibration, altho cumbersome, was deemed advisable owing to the considerable variations in bore in tubing of this diameter. The tube was then waxed, the lines and numbers (from zero to the limit of graduations from each end) were incised with a sharp steel point, and etched with hydrofluoric acid.

Assembly and Adjustment

The apparatus was assembled by attaching one hopper to the supporting frame, slipping the measuring tube between the concentric sleeves and the upper hopper over the measuring tube, and then attaching the upper hopper to the frame.

The measuring tube, which is free to move vertically through a distance of about two inches, was so placed that the central collar was in line with the bearings, and was temporarily fixed in position by cork wedges placed between the tube and the outer collars.



The hopper was filled with rape seed to the lower zero mark, the apparatus inverted, and the level of the seed noted. The tube was then moved up or down by trial until on inversion of the apparatus the seed coincided with both zeros. During this adjustment the standard loaf was removed.

The tube was then permanently fixed in position by filling the annular space between the tube and the sleeve with sealing wax.

Use of Apparatus

In use, the standard loaf is fixed permanently in one end of the apparatus, and sufficient rape seed is added to give a reading

of 30 cc. on the measuring tube. The test loaf is then inserted, the apparatus inverted, and the reading taken. The volume of the loaf is obtained by adding 420 cc. to the reading on the measuring tube. A blank reading is made between each two determinations. Duplicate readings of volume can be made within 2 cc.

The apparatus, as described, can be constructed in almost any laboratory, at a total cost for materials of approximately seven dollars.

Acknowledgment

The authors are indebted to the engineering department of the Manitoba Agricultural College for the construction of the supporting frame and the internal collar.

CALIBRATION OF LOAF VOLUME BOXES

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(Received for publication March 10, 1928)

The measurement of loaf volume by displacement of seed is subject to various errors. Three procedures are possible in finding the loaf volume.

First: Box volume (measured or calculated) minus volume of seed retained in box, equals loaf volume.

Second: By use of a constant volume of seed equal to the box volume, the overflow represents loaf volume.

Third: Placing the known volume of a standard object as the ordinate and the volume of the retained seed as the corresponding abscissa, it is assumed that the slope of the curve equals 1. Increments in the abscissa represent corresponding decreases in volume and vice versa.

The accuracy in the first method depends upon having the correct box volume; in the second upon the ability to maintain a constant seed volume; while in the third the correct determination of the volume of the standard object is of prime importance. All three of these procedures presuppose the determination and correction for the specific volume of seed due to the packing effect and inclusion of air which varies according to the conditions.

The various volume boxes now being used have been found by the writer to vary as much as 10 per cent. This error was often due to incorrect standards. Often proper corrections were not made for the specific volume of the seed. The following method has been found exceedingly satisfactory.

The density of the rubber in small toy balloons was determined. This was done by weighing, then cutting into small pieces and noting the water displaced. The density thus obtained was 1.234.

A balloon for the calibration was then weighed and filled with water. This is easily done by inserting a glass tube in the opening of the balloon and connecting it with a faucet. When the proper amount of water is in the balloon, it is taken from the faucet. By holding the glass tube perpendicular, it may be observed when all of the air is expelled. Likewise, air bubbles in the balloon can be detected by holding a strong light on the opposite side. The opening of the water-filled balloon is tied with a string, the balloon is dried, and weighed. Table I gives the data as recorded.

TABLE I

Weight of rubber	7.4 gm.
Weight of filled balloon	1253.0 gm.
Weight of water	1245.6 gm.
Determined density of water	1.000
Calculated volume of rubber	6.0 cc.

It is essential that the density of the water be determined. Volume of balloon and water equal

$$\frac{1245.6}{1.0} + \frac{7.4}{1.234} = 1251.6 \text{ cc.}$$

The filled balloon is now placed in the box, which is then filled with seed and the top levelled off with a straight stiff-edged ruler. The volume of the remaining seed is plotted as the abscissa and the corresponding volume of the filled balloon and rubber V_1 as the ordinate. These are 2152 and 1251.6 cc. respectively.

To ascertain the increase in volume of the rubber when stretched, its thickness was measured. This measurement gave an average of 0.12 mm. The calculated diameter of the sphere of water of 1245.6 cc. volume equals 13,3493 cm. $\frac{dV}{dD}$ equals $1/6\pi 3D^2$, where V equals volume of sphere and D equals the diameter. Solving, we find 0.12 mm. to make 7 cc. difference. It therefore appears that the increased volume due to the stretched rubber is

1 cc., or negligible. Thus, one point has been established. It is very desirable to have this point close to the minimum volume of the loaves to be measured.

More water was added to the balloon. If possible, it is desirable to have enough water added so that the volume V_2 of the balloon and the rubber will equal the maximum volume of bread to be measured.

In a similar manner a second point was determined. The curve given by these two points gave very accurate readings. By calibrating twice and thus locating two points, the specific volume and packing effect of the seed are corrected for under the conditions of measurement. By making a calibration at a minimum and a maximum volume, more accurate readings are assured. After securing this calibration curve, any object of definite physical dimensions may be measured and the correct volume stamped thereon for future use. If two such objects are measured and stamped, one at a minimum and the other at a maximum volume, the packing effect of the seed will be corrected for. These two stamped objects can be kept as standards for future use.

THE ACTION OF PHOSPHATIDES IN BREAD DOUGH[1]

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The Action of Yeast in Dough Development

The use of yeast to leaven bread is older than the art of writing, but our knowledge of the physics and chemistry of the process is still fragmentary. The observation that the foam structure of a fermenting dough could be preserved by baking is very old, but all the factors causing the difference between the rather heavy texture of bread from an unfermented dough and the fine, silky texture from a dough correctly fermented, have not yet been determined. Factors which have been suggested are: The development of acid by the yeast; the action of proteolytic and diastatic enzymes in the yeast and in the flour; the action of phytase; and the mechanical action of stretching of the gluten strands. Jessen-Hansen (1911) called attention to the importance of hydrogen-ion concentration, and the function of acids produced during fermentation in the production of good bread. This seems to stand alone as the one fully proved effect of fermentation in bringing the dough into a physical condition to make bread of fine silky texture—the "development" of the dough, as it is called.

Among other factors, the physical action of stretching during the preliminary rising of the dough followed by a packing together when it is worked down, as suggested by Swanson and Working (1926), seems to have a sound theoretical basis.

The action of the proteolytic enzymes is still largely a matter of conjecture, as no increase in free amino groups has been shown in dough fermentation of normal duration. However, in the long fermentation given cracker doughs, Johnson and Bailey (1924) showed a marked decrease to occur in protein precipitable by copper and silver salts. It is probable that the initial action of proteolysis consists in breaking apart molecular aggregates, and perhaps altering molecular orientation. This could easily cause a profound change in the physical properties of the proteins without any change in the chemical identity of their molecules.

[1] Contribution No. 34, Department of Milling Industry. Read before the meeting of the American Association for the Advancement of Science, December 28, 1927, Nashville, Tennessee.

Unless added in abnormal quantities, the diastatic enzymes have been shown not to have any action other than the production of additional sugar to serve as food for the yeast. The dextrins, which are formed as the first step in diastatic action, may have a beneficial effect on the keeping quality of the bread.

No effect in dough development has been traced directly to the action of phytase, but the phosphates produced may be useful as yeast food.

The alcohols and glycerine produced during fermentation do not seem to have been studied as possible factors in the development of the dough.

Making Bread Quickly

If the factors which produce the effect called development of the dough can be artificially imitated when the dough is mixed, it should be possible to dispense with the fermentation period. The quality of bread produced then becomes a measure of the accuracy of the imitation.

Two methods of making bread without long fermentation have attained some commercial importance. The Fleischmann "No Dough Time" method consists in using a large percentage of yeast and allowing the dough to rise in the pans at an unusually high temperature, 40 to 45 degrees C. This high temperature itself softens the gluten, and the difference in the products of fermentation at high temperature may also have an important effect. In the American Institute of Baking "No Dough Time" method, lactic acid and powdered buttermilk are added, taking the place of the acids normally produced during fermentation.

Neither of these methods has been entirely satisfactory, as the softening of the gluten by high temperature seems difficult for the average baker to control with sufficient accuracy, and the addition of acid alone will not give the combination of large volume, fine texture, and keeping qualities that are obtainable by fermentation.

The method of mechanical fermentation of dough developed by Swanson and Working produces bread of excellent volume and texture, but requires a dough too slack to be easily handled by ordinary bakery machinery.

Substances Producing Effects Similar to Overfermentation

During some work on the effect of lipoids on gluten quality, it was observed by the author that one-half of 1 per cent of phosphatide added to ordinary bread dough produced an effect in the resulting bread similar to that of overfermentation. It was also noted that phosphatide would, to some extent, take the place of the longer fermentation ordinarily necessary when large amounts of milk are used in dough.

The action of oxidizing agents in simulating the effect of overfermentation has been used by Blish and Sandstedt (1927) in testing fermentation tolerance of doughs.

Experiments on Artificial Dough Development

The idea presented itself that it might be instructive to determine the value of these substances, and also of alcohol and glycerine, two of the most important by-products of fermentation, in replacing fermentation. To carry out this experiment the regular bread formula of this laboratory was used:

Flour	340 gm.
Salt	5 gm.
Sugar	15 gm.
Yeast	10 gm.
Lard	5 gm.
Water	According to absorption

The substance or substances to be tested were added, usually by solution in the water used. The dough was mixed 15 seconds at low speed and 75 seconds at high speed. When the commercial type open pan was used for baking, the dough was placed on the table, covered with a cloth, and after ten minutes molded, placed in the pan, and allowed to rise at 34° C. until ready for the oven. When it was desired to detect very slight differences, the cylindrical closed pan, developed by Swanson (1911) in this laboratory, was found most satisfactory. In this case the dough was rounded up in the hands and placed in the pan directly from the mixer.

Figure 1 illustrates the effect of very small amounts of phosphatide on bread dough. A solution or emulsion of a sterol in lecithin gave some evidence of being slightly better than lecithin alone. In egg yolk we probably have such an emulsion of sterol in lecithin.

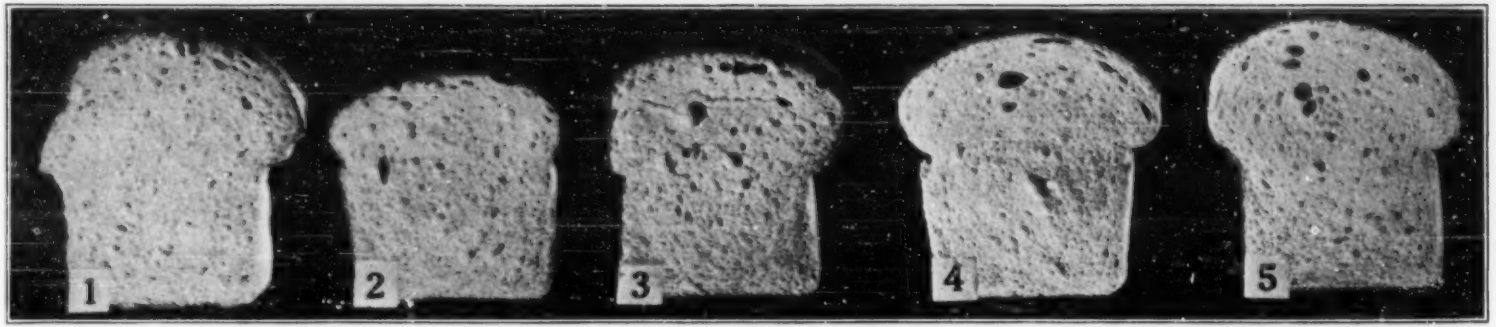

Fig. 1. (above) Effect of Phosphatide
All mixed 1½ minutes.
All except No. 1 panned 10 minutes after mixing.

Loaf No.	Loaf volume	Texture
1. Fermented 3 hr. at 32° C. before panning	2650	96
2. No addition	2340	95
3. 1 gm. egg yolk	2460	98
4. 1 gm. egg yolk, 0.75 ml. 90% lactic acid	2820	98
5. 0.1 gm. lecithin, 0.75 ml. 90% lactic acid	2905	96

Fig. 2. (below) Effect of H-Ion Concentration when Egg Yolk is Added
All contain 1gm. egg yolk; mixed 1½ minutes; panned 10 min. after mixing.

Loaf No.	Initial pH of dough	Loaf volume ml.	Texture
1. 75 ml. 0.1 N. NaOH	7.0	2190	93
2. 50 ml. 0.1 N. NaOH	6.6	2280	95
3. 25 ml. 0.1 N. NaOH	6.2	2340	97
4. No acid or alkali added	6.0	2460	98
5. 0.5 ml. 90% lactic acid	5.4	2475	98
6. 0.75 ml. 90% lactic acid	5.0	2570	98
7. 1.0 ml. 90% lactic acid	4.8	2660	98

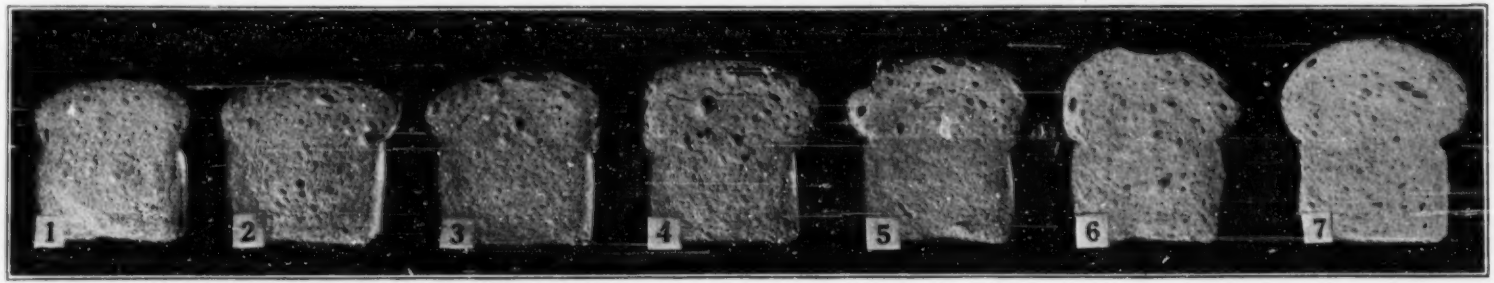

Fig. 3. (above) Effect of Alcohol
All mixed 1½ minutes; panned 10 minutes after mixing.

Loaf No.	Loaf volume ml.	Texture
1. No addition	2340	95
2. 3 ml. alcohol	2630	95
3. 0.75 ml. lactic acid	2730	98
4. 0.75 ml. lactic acid and 3 ml. alcohol	2820	98
5. 0.75 ml. lactic acid and 1 gm. egg yolk	2820	98
6. 0.75 ml. lactic acid, 1 gm. egg yolk, and 3 ml. alcohol	2840	98

Fig. 4. (below) Effect of Glycerine
All mixed 1½ minutes; panned 10 minutes after mixing.

Loaf No.	Loaf volume ml.	Texture
1. No additions	2340	95
2. 1 ml. of glycerine	2610	96
3. 0.75 ml. lactic acid	2730	98
4. 0.75 ml. lactic acid, 1 ml. glycerine	2755	98
5. 0.75 ml. lactic acid, 1 gm. egg yolk	2820	98
6. 0.75 ml. lactic acid, 1 gm. egg yolk, and 1 ml. glycerine	3090	98

Figure 2 illustrates the effect of hydrogen-ion concentration when egg yolk is used, showing a steady increase in volume as the initial pH of the dough is decreased from 7.0 to 4.8. The volume and texture of the loaf are given.

In Figure 3 is shown the increase in volume produced by the addition of alcohol, whether used alone or in addition to acid and egg yolk. Figure 3 gives the details of treatment. It was also observed that when only alcohol and lactic acid are used, bread is decidedly injured by the omission of lard from the formula. This is not the case when egg yolk is used.

Glycerine has an effect similar to that of alcohol, as is shown by Figure 4.

Results very similar to those produced by acid and phosphatide were obtained when acid and an oxidizing agent were used. Potassium bromate, potassium iodate, potassium chlorate, sodium chlorate, hydrogen peroxide, and ammonium persulphate were used at the rate of 0.003 to 0.006 per cent of the flour used. Novadelox B, a commercial preparation of benzoyl peroxide, was used at the rate of 0.01 to 0.02 per cent. Nitrogen trichloride, and free chlorine were used in undetermined amounts. When clear flour, and mixtures of graham and white flour were used, and when 4 to 6 per cent milk solids were used, a decided improvement was obtained by adding both phosphatide and an oxidizing agent.

Because of their similarity to lecithin in surface phenomena, the addition of saponin and of soap was tried. Saponin and lactic acid gave bread fully equal to that produced by lecithin and lactic acid, but soap had little effect, probably because of its decomposition by the acid used.

Search for Cause of Results Obtained

The effect of acid on dough is presumably due to its effect in increasing the imbibition of water by the gluten. To differentiate clearly between the effect of acid and that of the other substances used, it was first necessary to find the optimum acid concentration. Results shown in Table I indicate that this is about 1.0 ml. of 90 per cent lactic acid for the hard winter wheat flour used throughout this experiment. This produces an initial pH of 4.8 in the dough.

Then, with 1.0 ml. of lactic acid in each loaf, the effect of the addition of egg yolk, alcohol, and potassium bromate was observed, as shown in Figure 5. In each of these loaves, the dough was

reduced to 540 gm., as the normal 600 gm. produced loaves too large for the pans used.

TABLE I

OPTIMUM ACID CONCENTRATION

All mixed 1½ minutes; panned immediately. 90 per cent lactic acid added.

	Loaf volume	Texture
ml.	ml.	
0.75	1960	96
1.00	2020	96
1.25	2000	95
1.50	1970	94

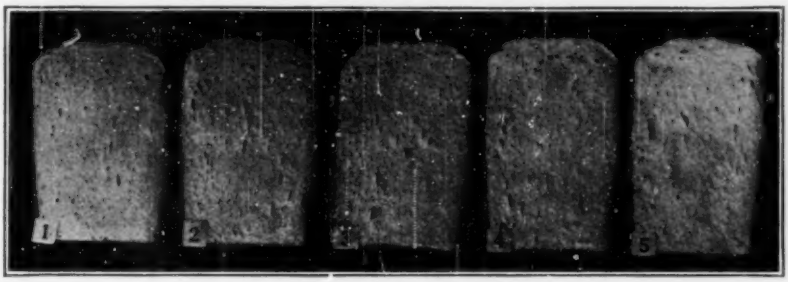

Fig. 5. Effect of Phosphatide, Alcohol, and Oxidizing Agents
All mixed 1½ minutes; panned immediately.
Each contains 1.0 ml. 90% lactic acid.

Loaf No.	Loaf volume ml.	Texture
1. No additions	1920	96
2. 1 gm. egg yolk	1945	97
3. 2 gm. egg yolk	1955	97
4. 3 ml. alcohol	2040	98
5. 0.01 gm. $KBrO_3$	2055	99

As these substances used with acid produce a greater volume and better texture than can be obtained with any amount of acid alone, their action upon the dough must be different from that of acid, even tho the results in the baked bread are similar.

It has been noted above that 0.1 gm. of lecithin and 0.75 or 1.0 ml. of lactic acid give, without fermentation other than in the pan, bread as good as fermented bread. This amount of lecithin is 0.03 per cent of the flour used, and corresponds to about 0.001 per cent of lipoid phosphorus. But there is normally in flour 0.006 to 0.01 per cent of lipoid phosphorus, which must be present chiefly as phosphatides. Of all the constituents of flour, these phosphatides are probably the most likely to be affected by minute amounts of oxidizing agents. It was therefore thought worth while to study the effect of different treatments upon these phos-

phatides. Accordingly, doughs before fermentation, after fermentation, and without fermentation but with the addition of various "developing" agents, were rubbed up with water in a mortar, made up to 500 ml., and extracted for 30 minutes. The solutions were then centrifuged, filtered, and an aliquot evaporated to dryness in vacuo. The residue was extracted with alcohol and ether, and the combined extracts were evaporated and taken up with chloroform. Phosphorus was determined on the chloroform-soluble material, as given in Table II.

TABLE II

WATER-SOLUBLE-PHOSPHATIDE PHOSPHORUS IN DOUGHS

Treatment of Dough	Additions		Water-soluble-phosphatide phosphorus Per cent
	Lactic acid	Other	
Mixed 1½ minutes	None	None	0.0006
Mixed 1½ minutes	0.75	0.01 gm. K BrO_3	0.0006
Mixed 1½ minutes	0.75	0.1 gm. saponin	0.0015
Mixed 1½ minutes	0.75	3 ml. alcohol	0.0008
Mixed 1½ minutes	0.75	1 ml. glycerine	0.0007
Mixed 1½ minutes (fermented)	None	None	0.0014
Mixed 9 minutes ("mechanically modified")	0.5		0.0007

The apparatus and technic had not been developed sufficiently to give these figures very high accuracy, but they are thought to be significant, at least in the larger differences. The determinations will be repeated with greater refinement of methods as soon as possible.

Theory of the Production of Good Bread

Good bread dough, when ready for the oven, is essentially a stable, extensible foam. A proper balance of viscosity and reduction of surface tension promotes foam formation. However, if the foam is to be stable, this is not sufficient; something approaching a gel structure must be present in the films of liquid, as occurs with soaps, saponin, the gums, and the proteins. In bread dough, the films forming the foam must be strong enough to support the large percentage of starch, extensible enough not to be ruptured by the over-rise, and thermo-stable enough not to break before baking temperature is high enough to fix them by coagulation. This requires a balance between extensibility or ductility, and tenacity or elasticity in the true physical sense of that word.

The most probable structure that has been suggested for wheat proteins is that of an interlacing meshwork of exceedingly fine fibrils or strands surrounded by a liquid phase of protein dispersed

in water. It is therefore suggested that the elasticity must be a function of the fibrils themselves dependent upon their tendency to return to their original shape after being stretched or deformed. Similarly, ductility must be a function of the surface forces binding the fibrils together and slippage of one fibril upon another sets up no tendency toward recovery.

These assumptions clearly explain the difference between the action of acid and the action of phosphatide on dough. The acid increases the swelling or water imbibition of the gluten fibrils, thus decreasing their elasticity or tensile strength. The phosphatide, on the other hand, spreads over the interfaces between the fibrils, allowing them to slide more readily upon each other; thus the ductility of the mass of gluten is greatly increased, tho there is little or no change in the individual fibrils. Both of these actions are necessary to produce the best bread from normal bread flours.

As has already been stated, ordinary flour contains much more phosphatide than is necessary properly to develop the dough when acid is added. Apparently this phosphatide is so constituted that it cannot spread over the interfaces between the gluten fibrils. It seems probable that the action of oxidizing agents consists in breaking up these combinations so the phosphatide can be dispersed in the water present. Fermentation must have this action, as well. Alcohol, glycerine, and saponin probably increase the dispersion of phosphatide, and possibly also affect the surface forces directly. The sugars produce surface effects similar to those of glycerine, and this action may explain part of the increase in loaf volume when the amount of sugar is increased above that necessary as yeast food. The organic acids also doubtless produce some of the surface effects entirely aside from their imbibitional effect.

Salts decrease the dispersion of phosphatides in addition to their well known action in decreasing the swelling of gluten by acid. The presence of a large amount of ash salts in a flour may render the use of additional phosphatide more effective in developing the dough than the use of an oxidizing agent alone.

These studies open the way for a much better knowledge of the physico-chemical effects of the aging and bleaching of flour; and an understanding of two distinct effects of fermentation should enable the chemist to better analyze the possibilities and shortcomings of the flour he is testing. The present conventional

baking test, with its large amount of yeast and short fermentation period, copies the method used by the baker to coddle a weak flour. It penalizes a very strong flour as much as it does a very weak one. The suggestion by Blish, that an oxidizing agent be added, seems to be a step in the right direction. Perhaps it would be possible to reduce the variable factors in the test by omitting the fermentation period and adding acid and a somewhat larger amount of oxidizing agent.

Practical Applications

Artificial development of dough produced by the combined effects of acid and phosphatide has given much better results than any no-dough-time method previously published.

The acid used may be any one not harmful to health, but the use of lactic acid is recommended, with acetic acid as a possible substitute. The amount should be adjusted to the buffer value and acidity of the dough ingredients, so the hydrogen-ion concentration of the dough will be within the range pH 4.8 - 5.1. This may be arrived at roughly by using as much acid as is possible without giving an acid flavor to the bread. Sodium chlorate is recommended, but any oxidizing agent which does not leave a deleterious residue seems to be satisfactory. Most flours seem to tolerate considerable variation in the amount of oxidizing agent. With some flours, usually those with a high ash, and doughs containing considerable milk solids, better results are obtained by using additional phosphatide as well as the oxidizing agents.

Milk, potato flour, dextrin, malt, whole wheat flour, and other materials used to improve the nutritive value, flavor, moisture-holding capacity, and other properties of fermented bread, may be used with equal success with this method.

This method is being used successfully by farm women in their own kitchens, the acid being supplied by unstandardized buttermilk or sour milk, roughly measured, and the phosphatide by adding one egg yolk to any batch of from two to ten loaves. A tablespoonful of the solution made by dissolving a 5-grain potassium chlorate tablet in a cup of water may be added for increased volume.

The following formula has been successfully used in a commercial bakery, loaves scaled at 17 ounces having an average volume of 134 cubic inches.

Flour	196 pounds
Sugar	1 "
Sweetened condensed milk	6 "
Malt extract	4 "
Arkady	1 "
Salt	3½ "
Yeast	6 "
Lard	2¾ "
Water	129 "
Lactic acid 85%	7 fluid ounces
Sodium chlorate	1 /7 ounce

(Dissolve one ounce of sodium chlorate in seven pints of water and store in a glass jug. Use one pint for each barrel of flour.)

Mix from 10 to 12 minutes at high speed. Divide and round up immediately. Rest from 15 to 20 minutes and mold. Give full proof at 90° to 100° F., preferably starting at 100° and allowing the temperature to fall to 93° by the end of the proof.

The addition of ½ pint to 1 pint of egg yolks to the above mixture brings about a decided improvement with certain flours, especially those with high ash.

Summary

At least two distinct changes are brought about in dough during its development by fermentation. Both of these are required to produce the proper balance between tenacity and ductility necessary for the production of the best bread. One of these changes is the reduction of the tensile strength of the gluten strands caused by acid through its effect of swelling the strands by increasing their absorption of water. The other change is the increase in ductility of the dough, caused by substances such as phosphatides through a lubricating effect on the gluten strands, allowing them to slip more readily upon each other.

By adding acid and also egg yolk, or an oxidizing agent that will cause the liberation of phosphatide, thus artificially bringing about these two changes, either the housewife or the commercial baker can produce finished bread and buns of the best quality, two hours after the mixing is started.

This sharp differentiation of two factors in dough development should make possible more intelligent testing of flour for quality and fermentation tolerance, and a more intelligent study of the problem of flour maturing and bleaching.

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BOOK REVIEW

Milling Studies, by Edgar S. Miller. Published by the National Miller, Chicago.

This is an elementary book and intended for the active mill operator. However, as a general outline of the actual milling operation and the underlying principles of the processes employed, it will also serve as a general textbook for those who do not wish to go into intricate details and will be satisfied with the broader aspects of this subject.

Mr. Miller has been an operative miller for a considerable number of years and has the faculty of recording his observations in such a way that it is a pleasure to follow him in his descriptions and discussions.

The book is divided into two parts, viz: The Elements of Scientific Milling and The Analysis of a Flow Sheet. In the former, the author deals with the scientific principles underlying the various milling operations and describes the different processes through which the wheat travels during its conversion into flour and offal products. The latter part treats with the entirely mechanical process of breaking, classification, reduction, separation and purification.

The author being a miller proves himself on entirely safe ground where he treats with the mechanical phase of the milling process, and in this respect nothing but praise can be said about his book. However, this cannot be said when he deals with purely chemical, physico-chemical, or biochemical subjects, to which a considerable part of his work has been dedicated. There are so many inaccuracies, half truths, and errors in this part of his work that it detracts from the usefulness of an otherwise valuable book.

It is also regrettable that the chapter on flour bleaching is decidedly incomplete. Bleaching is now one of the most important phases in the manufacturing of flour and the fact that it is able to make or to break the flour quality, entitles it to an extensive discussion in any book of this kind.

Notwithstanding its faults, the book will fill the need for an elementary work on the subject.

J. T. FLOHIL